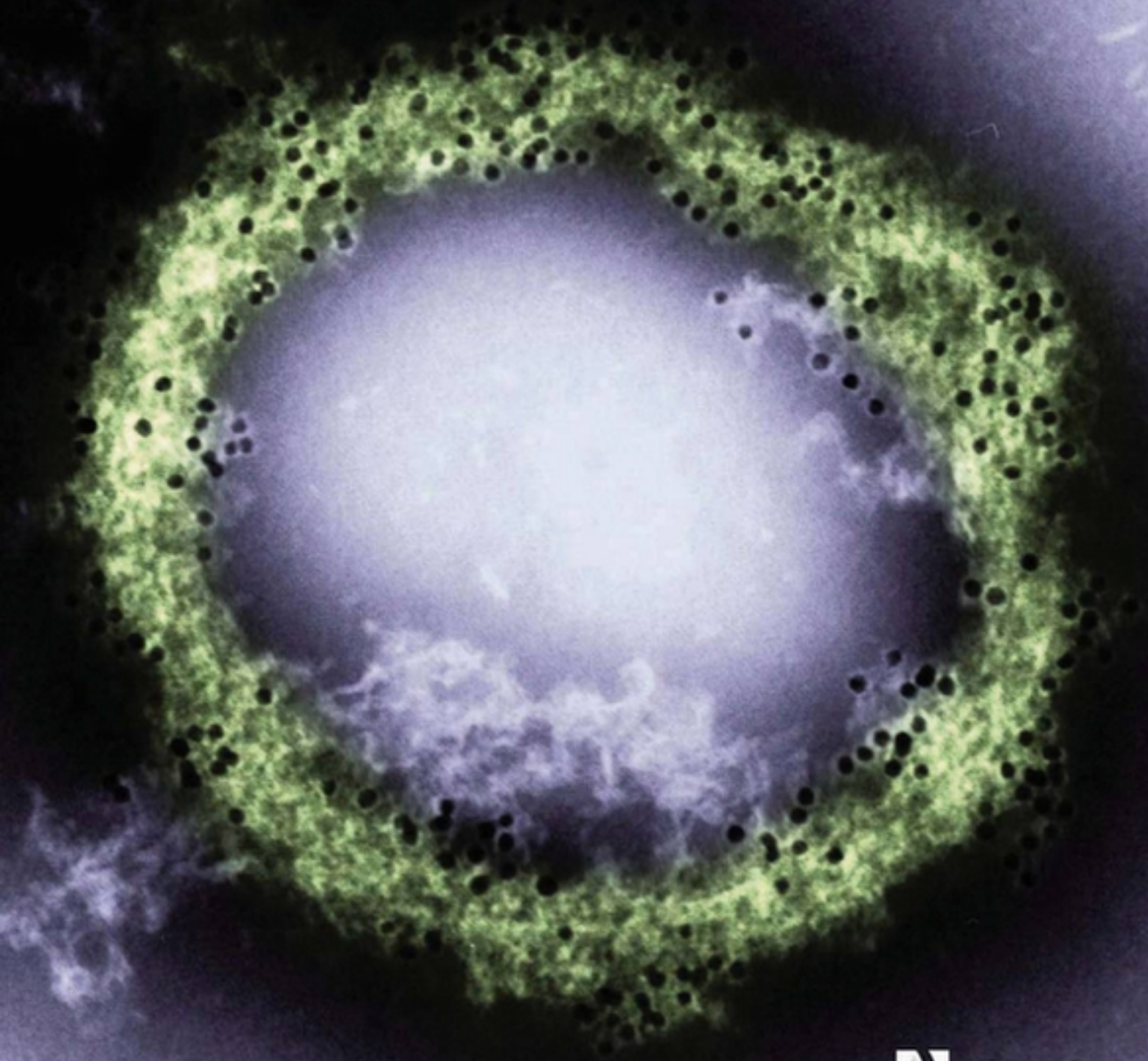


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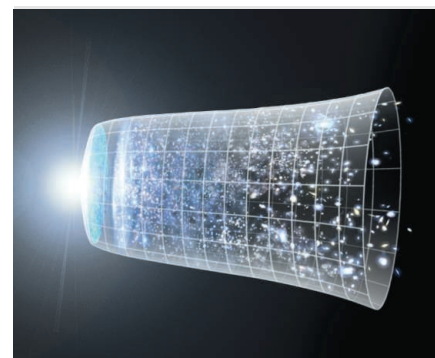
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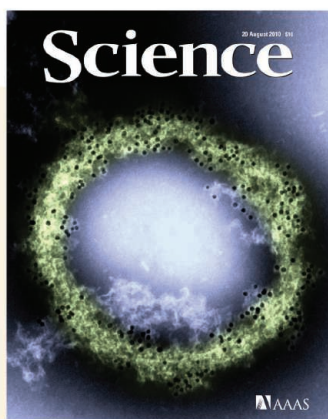
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Immuno-electron micrograph of a plastid-dividing ring (diameter ~500 nm), a structure required for chloroplast division, isolated from the unicellular alga *Cyanidioschyzon merolae*. Immunogold particles (black dots) indicate localization of the glycosyltransferase protein PDR1 (protein dividing ring 1), which forms a ring with carbohydrates that constricts to physically divide the chloroplast. See page 949.

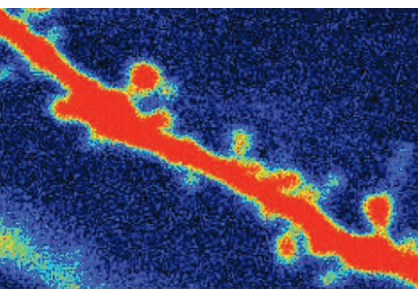
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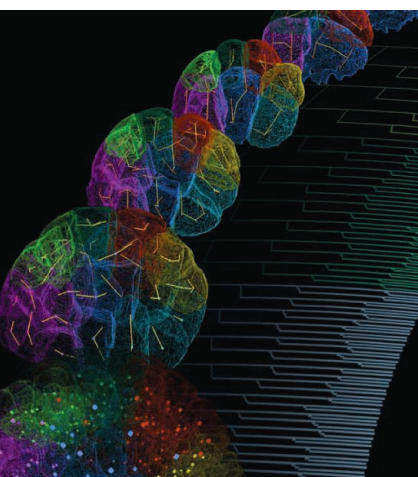
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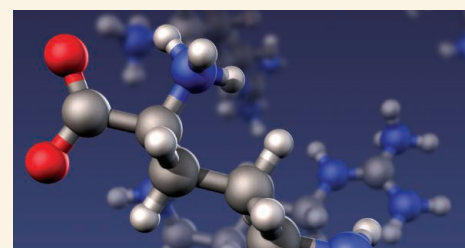
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ADVANCING SCIENCE. SERVING SOCIETY

Nurturing Young Scientists

THE UNITED STATES NATIONAL ACADEMIES HAS CONVENED A BLUE-RIBBON COMMITTEE TO RECOMMEND ways to keep research universities healthy.* It will be important for this panel, and for universities, to recognize the value of early career scientists—the transformative discoveries they will make and the students they will influence—as key to America's future. The good news is that federal and private funding for breakthrough research and innovative approaches in science education are growing for early career researchers. Now, universities must step up to the plate and create frameworks in which interdisciplinary research and synergistic teaching can thrive. Achieving these goals will require a fundamental cultural change.

Academia must do more to nurture early career scientists by reevaluating promotion and tenure policies. The criteria should affirm and reward cross-boundary collaborations that are essential to breakthrough science. This entails redefining what constitutes original and independent contributions to a research agenda that involves multiple partners, and rewarding those who forge robust interactions among groups.

Most universities have a department-centric organization. Each department's faculty judges its peers according to the norms of their own discipline, a perspective that makes it difficult for interdisciplinary faculty to receive a fair hearing. Therefore, universities need to give science departments a stake in the success of collaborative ventures by rewarding individuals and departments that build bridges to other groups, are community-minded, and share credit. Important departmental benefits include broadened research boundaries, new tools for research, more diverse faculty and students, research funds from non-traditional sources, and curricula that engage more students.

Currently, a handful of schools address collaborative/interdisciplinary research for early career faculty. At the University of Wisconsin, cross-departmental committees oversee progress for untenured faculty in both research and teaching. At Brandeis University, there are explicit expectations that collaborative/interdisciplinary research is a value-added contribution to a candidate's tenure profile. And at the University of Arizona, written agreements among department heads, deans, the provost, and newly hired faculty define the role of collaborative/interdisciplinary research in tenure and promotion. The common policy denominator is early, close, and continual communication among untenured and senior faculty and institutional administrators. This constant consultation is essential to overcoming bias and sustaining agreed-upon, progressive evaluation pathways.

Beyond a proclivity for innovative research, early career professors often seek to integrate their research and teaching, eager to bring the vitality of their own science into the classroom to engage students. Unfortunately, great teaching skills are still considered to be of secondary value to career success at research universities. This long-standing problem must be solved. Carl Wieman, the Nobel Prize-winning physicist recently nominated by President Obama to be the Associate Director for Science in the Office of Science and Technology Policy, testified before the U.S. Senate in May that "To maintain U.S. economic competitiveness and leadership in innovation, we need to also have leadership in STEM [science, technology, education, and mathematics] education." The National Academies and several private foundations have an annual institute where research university faculty teams collaborate to improve student learning in introductory college biology classes. The key here is building a critical mass of individuals for long-term cultural change within a given department, something that can only be sustained through appropriate tenure and promotion policies.

It is imperative to grow our economy through global leadership in science. To accomplish this goal, the National Academies panel must look well beyond funding issues, toward creating incentives that foster new collaborative communities of early career scientists and the students they teach.

— James Gentile and Sherwood Boehlert

10.1126/science.1196350

*J. Mervis, *Science* 329, 126 (2010).



James Gentile is President and CEO of Research Corporation for Science Advancement, Tucson, AZ. E-mail: gentile@rescorp.org



Sherwood Boehlert is a retired member of the U.S. House of Representatives; he served on the Science Committee throughout his 24-year congressional career and was its chairman from 2001 to 2007.



**1200 New York Avenue, NW
Washington, DC 20005**
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6581, FAX 202-371-9227
**Bateman House, 82-88 Hills Road
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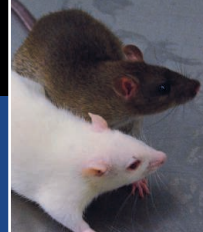
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GULF OIL SPILL

After Outcry, Oil Data Inches Into the Open

Fisheries scientist James Cowan wasn't put off when a private consulting firm contacted him about helping BP with its oil spill research—indeed, he welcomed the opportunity. “I don't want [BP] to take samples that give them the wrong information, so I'd just as soon help,” says Cowan, a professor at Louisiana State University (LSU), Baton Rouge. For instance, when a team under the direction of the National Oceanic and Atmospheric Administration (NOAA) and BP went out with an acoustic instrument to verify the oil plumes reported by academic scientists, they came back empty-handed—until Cowan told the consulting firm that the team was likely using frequencies that couldn't resolve the oil droplets at the depths they were searching. He suggested a different technique, and “now no one can deny the existence of the plumes, which many did for a very long time,” he says.

But Cowan has been careful to give his advice pro bono. One reason: He's married to a restoration scientist at NOAA. That agency leads the so-called Natural Resource Damage Assessment (NRDA), the legal pro-

cess during which the government and BP collect evidence on the extent of the oil's impact—evidence that will factor, eventually, into BP's liability. But Cowan was also uneasy about the consulting contract the firm offered him, which would have banned him from discussing or publishing any data collected on their dime for up to 3 years. “My biggest concern [was] that the data we'd collect under direct funding would be tied up in litigation,” he says.

Now, in a largely unexpected and welcome move, BP has revised its contracts to remove these restrictions. And with NOAA relaxing its own restrictions on publishing assessment data, scientists are hopeful that the NRDA process will be less adversarial than they'd feared. A sample contract provided to *Science* by BP allows signers to publish “written research papers, presentations and similar documents reporting any environmental data obtained or produced” as a consultant after giving BP 30 days' notice and a copy of the intended publication.

The change comes after a public cry of

outrage sparked by a damning story in the 16 July Mobile, Alabama, *Press-Register* that blasted BP for “buying up” gulf scientists by the department. Cary Nelson, president of the American Association of University Professors, wrote an editorial in *Inside Higher Ed* asking scientists not to sign—and urging university officials to prevent their faculty members from doing so. One LSU scientist who is consulting for BP received several threatening e-mails after being quoted in the press.

A group of scientists at the University of Southern Mississippi (USM) in Hattiesburg first agreed to consult for BP but backed out when they saw the contracts barring them from publishing. Last week, however, after BP lawyers came back with the new contracts, several of them signed on again, says Denis Wiesenburg, USM's vice president of research. “Our understanding is that any environmental data our faculty produce when they're working as consultants for BP will be made public,” he says.

That's quite a contrast to the scientific feuding that ensued after the oil tanker *Exxon Valdez* broke open on a reef in Alaska's Prince William Sound on 24 March 1989. “Shadow studies” were common, says Tom Brosnan, a NOAA environmental scientist who's worked on NRDAs for 11 years. The government, for example, would sample an area, and Exxon scientists

GULF OIL SPILL

The Case of the Missing \$470 Million in BP's Promised Research Fund

It was a rare piece of good news in the early, dark days of the Gulf of Mexico oil spill. On 24 May, as scientists fretted about what the oil gushing out of BP's blown Macondo well might do to deep-sea corals, wetland birds, and young fish, BP pledged \$500 million over the next 10 years to support independent research to answer such questions. But just 3 weeks later, a presidential directive put the money on hold. Now that 2 months have passed with few clues about the fate of the money, scientists are worried that what many saw as their best hope for a long-term, comprehensive research plan will disappear along with the oil slick.

BP announced the \$500 million Gulf of Mexico Research Initiative after a 19 May meeting with academic and government

scientists convened by the White House Office of Science and Technology Policy. On 15 June, after a second meeting at Louisiana State University (LSU) sponsored in part by the National Oceanic and Atmospheric Administration (NOAA) and attended by 200 scientists, BP announced that \$25 million would go immediately to four consortia of gulf universities, which could then distribute the funds to individual projects. (BP promised an additional \$5 million to a fifth consortium in early July.) That same day, BP named a crack science advisory board, headed by former National Science Foundation Director Rita Colwell, and announced its intent to launch a request for proposals.

But the next day, it all ground to a halt, thanks to a paragraph at the bottom of a White

House fact sheet on the \$20 billion escrow account BP would use to pay future claims. The Administration, referring to the \$500 million pledge, directed BP to “work with governors, state and local environmental health authorities to design the long-term monitoring program to assure the environmental and public health of the Gulf Region.”

But BP's original approach “would have been a good thing,” says Kevin Wheeler, vice president of the Consortium for Ocean Leadership, a Washington, D.C.-based nonprofit representing oceanographers. “It was a more coordinated science initiative than what the government has put out. ... Now we're 6 to 8 weeks out [following the decision to involve governors], and there's no plan” to distribute the rest of the money.



Astronomers' top priorities for the decade
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would follow—then claim different results.

Then, in August 1990, Congress passed the Oil Pollution Act, which laid out the current NRDA process. It also requires the environmental trustees—in this case, NOAA, several Department of the Interior agencies, affected states' environmental agencies, and possibly tribes—to give BP the opportunity to collaborate.

"It's better if you can agree with a responsible party on the basic facts of the case," says Brosnan. And so far the assessment process following the *Deepwater Horizon* disaster seems to be collaborative. During the first part of the NRDA, which involves tak-

ing baseline data and determining whether resources have been harmed, BP and the trustees have—for the most part—been working together, even sending scientists out on the same boats to take a single set of samples. "If you're out in a marsh assessing a shoreline, and you're on a boat together, and you agree on how [samples] are going to be collected, what that marsh looks like in that data, you're a lot further along."

After being lambasted with phone calls from a concerned public, and given that BP was seeing the data anyway, NOAA decided on 8 July that it would post its vetted data online and began doing so in a matter of

in this category, and so it isn't going online.

Nor is collaboration guaranteed to continue into the next phase of NRDA—quantifying the damage and planning restoration—which begins in the next few weeks, he cautions. The trustees and BP might disagree on which studies to do or how, or "there may be ... a time when we say, 'This isn't working for us, and we need to go separate ways.'" And because NRDA data may ultimately wind up in court, NOAA "must reserve the right of what can be released and when"—and that applies to data collected by academic scientists working as consultants for the trustees.

For now, however, the détente is a relief to the academic scientists who are eager to be involved in research that will determine how the region is restored, says USM's Wiesenburg. And that's the way it should be, says the LSU scientist who received threatening e-mails for signing BP's original contract. Academic scientists "can and, I believe, do keep the NRDA process honest and objective, ... [acting] as independent checks on the data and the inferences derived from the data," he says. "Their reputations as credible scientists are extremely important, ... [so] they have too much to lose by biasing their results or opinions, whether they are consulting for NOAA or for BP."

—LAUREN SCHENKMAN



Cooperating. BP, NOAA, and state of Louisiana scientists check marsh sediment for oil.

weeks, which Brosnan admits is "a very unusual situation for us." With this spill, he explains, "the public wants the information, the academic community wants the information, and we're trying to get it out as quickly as we can."

Brosnan says NOAA's open-data policy applies to "everything where we have an agreed plan and are sharing the data with BP." But he admits that some of the data collected during the NRDA process does not fit

Local academics suspect that state governors lobbied the White House because they were worried that the funds would leave the gulf region if any institution were eligible to send in a proposal. "I think people got concerned that if you did it like that, then the big boys, like Woods Hole and Scripps, that always seem to get a lot of the money ... would get the lion's share," says Michael Carron, the director of the Northern Gulf Institute, a NOAA cooperative institute in Stennis Space Center in Mississippi.

Denis Wiesenburg, vice president of research at the University of Southern Mississippi in Hattiesburg, thinks such parochialism was unfair—and unneeded. "The money ought to be devoted to the best science," he says. "We think our scientists would be very competitive for BP research funding." But now that the money is in the hands of the governors, he says, "it sounds like [distributing

it] is going to be a political rather than a scientific decision."

Over the past 2 months, academic scientists and administrators have heard nothing about the remaining \$470 million. The advisory panel established by BP is on the sidelines; it hasn't met since the money was announced, says Colwell. In an e-mail to academics last week, a BP ecologist involved in the research plan said, "As of late last week, we were still working with the various governors' staffs on a way forward that would be acceptable to all parties." Scientists from four Louisiana universities, hoping to help guide the state-level negotiations, sent a detailed research proposal to their governor's office but received no response. Three governors' offices contacted by *Science* failed to provide a comment in time for this article.

The silence has frustrated scientists in the region, especially given that many of them

were hopeful about BP's initial plan. "Right now, [the BP money] is where the scientific community sees the long-term data coming from," says Wiesenburg. Chris D'Elia, dean of the School of the Coast and Environment at LSU, says he was "very surprised and discouraged. ... We've really lost some wonderful opportunities here by failing to get a substantial funding opportunity out there for the academic community."

The situation isn't dire, says Carron: The \$30 million already distributed is a lot to spend. But the lack of a long-term funding strategy limits scientists' ability to plan research over several years and to hire staff or graduate students, he says. The delay has him and others wondering if the promised funding will ever materialize. "The important thing is that the money doesn't disappear now that they've got this thing capped."

—LAUREN SCHENKMAN

ScienceInsider

From the *Science*
Policy Blog

Postdocs at the University of California (UC) have voted to adopt a 5-year contract that would raise their pay by between 1.5% and 3% this fall and give them new protections. The UC system's 6500 postdocs represent roughly 10% of all U.S. postdocs. <http://bit.ly/ucpostdocdeal>

The National Science Foundation's governing body, the National Science Board, is exploring whether the foundation ought to be more receptive to **large, out-of-the-box research proposals** not solicited by its staff members. The new study will look at proposals larger than the traditional award size to investigators but smaller than major facilities. <http://bit.ly/nsftaskforce>

A flawed satellite sensor set to fly late next year may be able to record **ocean color**, *ScienceInsider* has learned. The sensor, VIIRS, was to fly on the NPOESS Preparatory Project mission next year, but in 2008 officials decided not to fix a flaw preventing accurate measurements. New tests by government engineers, however, suggest that the problem was less severe than thought. <http://bit.ly/colorsattellite>

The European Space Agency has published a road map of favored **space missions for physics** between 2015 and 2025. The program includes efforts to test the fundamental laws of physics, find gravitational waves, and obtain antimatter. <http://bit.ly/esaroadmap>

The **H1N1 pandemic** that started in the spring of 2009 is now officially over, the World Health Organization declared. Scientists believe the real toll is much higher than the 18,500 confirmed deaths. <http://bit.ly/h1n1over>

Nobelist Roald Hoffmann has called for a **boycott of an upcoming Eurasian chemistry conference** in Jordan because he suspects that organizers have deliberately excluded Israeli speakers. Organizers say that's not the case and that it's too late to reshuffle the lineup of speakers. <http://bit.ly/jordanconference>

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COGNITION RESEARCH

Investigation Leaves Field in the Dark
About a Colleague's Work

The Boston Globe dropped a bombshell last week when it reported that Harvard University cognitive scientist Marc Hauser was on leave following an investigation of his research by the university. Hauser is a popular teacher, successful author, and leading researcher on animal cognition. In work spanning 3 decades, he has produced notable insights into the richness and complexity of primate cognition that have helped erode notions of human uniqueness. More recently, his work on the evolutionary roots of morality, along with his writing and public speaking on the topic, have earned him something close to celebrity status.

So far, one paper co-authored by Hauser—a 2002 report in *Cognition*—has been retracted as a result of the investigation, and concerns have been raised about several others, including one published in *Science*. Hauser is the only author common to all of them.

But exactly what went wrong with Hauser's research is still not clear. Citing the need to protect privacy, Harvard has not made its findings public. The federal agencies that fund Hauser's work, citing their own privacy policies, would neither confirm nor deny any ongoing misconduct investigations. Hauser did not respond to requests for an interview.

Several of Hauser's former postdocs, students, and collaborators discussed the case with *Science* but declined to speak on the record. Some said they felt torn between a desire to set the scientific record straight and a reluctance to speak ill of someone they saw

as a brilliant thinker and caring mentor. "I fully believe the allegations," says one former lab member. "That said, I consider him the best adviser that I've ever had. He is a creative, energetic, and charismatic person."

Other researchers are frustrated by the lack of information. "Hauser's rights are being protected, the university's rights are being protected, but who's protecting the scientists working in this field?" asks Michael Tomasello of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. "This screws us up all over the place because we don't know what to cite and what not to cite; we don't know what we can believe and what we can't believe."

Even in the best of times, primate cognition is a controversial field, and researchers are often skeptical of the work of their colleagues, says Dario Maestripietri, a behavioral biologist at the University of Chicago in Illinois. "This work is so amenable to subjective interpretation," he says. "You're trying to make inferences about cognition by observing behavior. It's not something you can prove with hard evidence."

Before the *Globe*'s revelations, however, there were already hints that some of Hauser's colleagues had misgivings about his work. In a 1995 paper in the *Proceedings of the National Academy of Sciences (PNAS)*, Hauser and colleagues reported that cotton-top tamarins, a New World monkey, could recognize themselves in a mirror, a sign of self-awareness. Previously, only great apes and humans had been shown to pass the

ScienceNOW

Monkey puzzle. A Harvard investigation has raised questions about Marc Hauser's primate research.

mirror test. Extending the capacity for self-recognition to tamarins, a distantly related species generally assumed to have less cognitive firepower, made quite a splash.

Gordon Gallup Jr., the researcher at the State University of New York in Albany who developed the test (*Science*, 2 January 1970, p. 86), says he doubted Hauser's findings from the beginning. He had done extensive work with several monkey species and failed to find any evidence for self-recognition. Hauser eventually agreed to send Gallup the videotapes for the tamarin experiment. "When I sat and watched the tapes, I just couldn't believe it," Gallup says. "There was a total disconnect between the claims that were being made in the paper and what was depicted in the videotape."

Hauser and colleagues then repeated the tamarin experiment and reversed their original finding, concluding that tamarins do not recognize themselves in a mirror. They published this result in 2001 in the *American Journal of Primatology*. The *PNAS* paper, however, has not been corrected or retracted.

The retracted *Cognition* paper reported that cottontop tamarins can learn abstract rules about strings of syllables (for example, that *la ta ta* and *ni gi gi* have the same pattern, but *wo wo fe* does not). Human infants can make this kind of inference, which some researchers argue is tied to the uniquely human capacity for language.

That paper also met with skepticism early on. Its conclusions hinge on a single data point representing the number of monkeys that turned to stare at a loudspeaker when it switched from playing one sequence of syllables to another, an indication that the animals recognized the difference, says psychologist Kim Wallen of Emory University in Atlanta. That's not very convincing, Wallen says: "It's a high-impact conclusion based on low-impact data." Gerry Altmann, editor of *Cognition*, says the journal received an e-mail from Hauser on behalf of all three co-authors stating that "An internal examination at Harvard University ... found that the data do not support the reported findings. We therefore are retracting this article. MH [Hauser] accepts responsibility for the error." Altmann says no further explanation was given.

Editors at *Science* and at the *Proceedings of the Royal Society B* have been notified of problems as well, but in both cases Hauser and a co-author informed editors that they had replicated the experiments and

verified their original conclusions. *Proceedings* noted this in an addendum published in July. The original papers, both published in 2007, described the ability of various primates to understand gestures made by a human experimenter, such as pointing to a hidden source of food. This ability to infer another's state of mind was once considered to be uniquely human (although recent research suggests that dogs can do it). Again, Hauser's findings seemed to narrow the cognitive gap between humans and our primate cousins. The *Science* paper, for example, reported that not only chimpanzees but also rhesus monkeys and tamarins could interpret human gestures—though some researchers weren't convinced (*Science*, 7 September 2007, p. 1308).

According to Ginger Pinholster, spokesperson for AAAS, which publishes *Science*, the journal's editorial staff received a letter in June from first author Justin Wood, now at the University of Southern California in Los Angeles, informing them that "an internal examination at Harvard University determined that there are no field notes, records of aborted field trials, or subject identifying information associated with the rhesus monkey experiments." Wood's letter goes on to say that results from a replication of those experiments "matched those reported in the paper in terms of statistical significance." *Science* has sent the newly submitted data out for peer review and will decide on a course of action after receiving the reviewers' comments, Pinholster says. Harvard has told *Science* editors that it will comply with a request for more information, but it had not sent more details about its investigation as this issue went to press.

Whether Hauser's fault was merely sloppy record-keeping and perhaps a rush to publish splashy findings or something more serious may not be known for some time. Robert Seyfarth, a veteran animal-behavior researcher who, along with his wife, Dorothy Cheney, was Hauser's Ph.D. adviser at the University of California, Los Angeles, in the 1980s, says the couple never had any reason to suspect Hauser of deliberate misconduct during his time with them. "However, even at this early stage, Marc was developing a reputation as a young man in a hurry, and ... we repeatedly urged him to slow down and be more thoughtful and cautious in his work," Seyfarth says. Now Seyfarth worries that Harvard's silence will cause some researchers to judge Hauser's postdocs and students guilty by association. "This would not be fair or true," he says.

—GREG MILLER

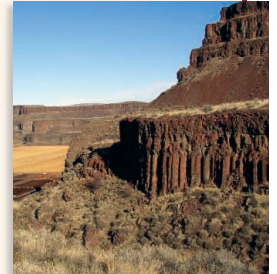
From *Science's* Online Daily News Site

Lasers Set Hearts Aflutter

Consider it a different kind of light therapy. Researchers have for the first time used a laser beam to control a heartbeat, an advance that provides a new tool for studying how hearts form and a possible step toward light-based pacemakers. <http://bit.ly/laser-hearts>

'Killer' Volcanoes Not Guilty

For years researchers have blamed quick, massive volcanic eruptions for numerous large-scale extinctions. That includes the largest one 250 million years ago that exterminated more than three-fourths of the species on Earth, an event so catastrophic it now carries the name the "Great Dying." There's just one problem: One of the biggest eruptions on record—an event that spewed 1.5 million cubic kilometers of lava over what is today southern Brazil, Paraguay, Uruguay, and southwestern Africa—didn't cause much damage at all. <http://bit.ly/killer-volcanoes>



Stars Steal Their Planet's Moons

Planning a honeymoon to a "hot Jupiter"? You might want to think again. It's bad enough that these giant planets orbit so close to their suns that they sizzle at temperatures exceeding Mercury's. But if an astronomer in France is right, you won't even have a romantic crescent to admire—because the planet probably has no moons. <http://bit.ly/no-moons>

How Tiny Drips Can Crumble a Building

It doesn't take a flood to destroy a building. Mere moisture over many years can do the same. Now, materials scientists have found a way to predict how moisture works its way through a given building and location, something that should lead to better assessments of the health of historic structures. <http://bit.ly/tiny-drips>

Read the full postings, comments, and more at news.sciencemag.org/sciencenow.

One-Two Punch Elevates Rats to the Knockout Ranks

Fans of the rat as a lab animal have watched in frustration as a parade of mice with specific genes deleted became available to researchers over the past 2 decades. While knockout mice have become models for many human diseases and developmental biology, scientists have longed for knockout rats because “the anatomy and physiology of the rat is closer to humans than is the mouse,” says Timothy Aitman, a molecular geneticist at Imperial College London and the Clinical Sciences Centre of the U.K.’s Medical Research Council. Time and again, however, knockout tricks that work in mice failed in rats.

That maddening species barrier has now been breached. Last week, a group led by Qi-Long Ying, a stem cell biologist at the University of Southern California in Los Angeles, reported online in *Nature* about having suc-

cessfully adapted to the rat the most widely used mouse knockout technique. This complements a different method for deleting rat genes described last year in *Science*. In addition to having two ways to knock out target genes, other groups have reported advances in techniques to produce transgenic rats as well as random knockouts that could be screened for interesting mutants. “It is an exciting time for rat genetics,” says Aitman.

The breakthroughs were a long time coming. The first knockout mice were created in 1989, using homologous recombination of embryonic stem (ES) cells—a technique that netted a trio of researchers the 2007 Nobel Prize in physiology or medicine. It relies on creating a DNA string that can replace or disable a target gene. Inserted into ES cells, the substitute DNA is incorporated into some of the cells as they proliferate. Adding the cells to an early embryo implanted in a surrogate mother results in some pups carrying

one copy of the original gene and one copy of the altered genetic material. Selective breeding can deliver mice lacking the target gene. Researchers have used homologous recombination to produce several thousand lines of knockout mice—but they could not apply it to rats because no one could find a way to culture rat ES cells.

The lack of rat knockouts has been a major impediment to biomedical research, says Aitman, who describes the rat as “an exceptional model for human disease.” The rat heart, for example, beats about 300 times a minute, which is much closer to the human average of 70 than is the mouse heart rate of up to 700 beats a minute. The patterns of electrical signals in rat and human hearts are also similar. The advantages even extend to behavioral studies, because rats, like humans and

genes in fruit flies and zebrafish (*Science*, 24 July 2009, p. 433). The zinc finger nuclease (ZFN) technology relies on artificial proteins that, when injected into an early embryo, recognize and cut specific DNA sequences. The genetic change is passed to offspring.

Meanwhile, Ying’s group solved the rat ES cell problem: A new culture medium they developed for mouse ES cells worked for rat ES cells as well. Once they could reliably culture rat ES cells, Ying says, it was a matter of tweaking the homologous recombination technique, as they reported online in *Nature* on 11 August, generating a line of rats missing the *p53* tumor-suppressor gene.

Each method has advantages. Compared with other available techniques, “using ZFN technology takes less time and fewer resources,” says Roland Buelow, a molecular immunologist at Open Monoclonal Technology in Palo Alto, California, and a co-author of the *Science* paper. Ying explains that homologous recombination promises greater flexibility in restricting gene modification to specific tissues or to a chosen developmental period. “Researchers have full control of the type of mutations they want to make,” he says.

There is no doubt about pent-up demand for the rat techniques. The ZFN method, available for just over a year, “has been quickly taken up by researchers across the world,” says Aitman. Geurts’s group has already produced 54 of a planned 100 knockout rat lines that will be used to probe genes implicated in hypertension and renal disease.

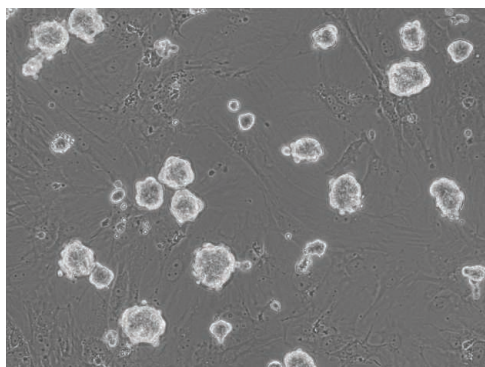
There have been other advances as well, particularly in the efficiency of transposons. At least two groups, one at Fudan University and one at Transposagen Biopharmaceuticals, a biotech start-up in Lexington, Kentucky, are using a transposon known as piggyBAC to generate large numbers of random rat knockouts. And Zsuzsanna Izsvák of the Max Delbrück Center for Molecular Medicine in Berlin and colleagues described in the June issue of *Nature Methods* what amounts to a transposon shortcut. Instead of breeding rats and screening for interesting phenotypes, they insert an improved version of a transposon called Sleeping Beauty into spermatogonial stem cells and then screen the cells for gene expression in tissue culture before they are used to produce transgenic rats.

Clearly, lab-rat lovers needn’t envy their knockout-mouse colleagues much longer.

—DENNIS NORMILE



KO’d at last. In a first, researchers got knockout rats (white animal, left) by manipulating embryonic stem cells (right).



cessfully adapted to the rat the most widely used mouse knockout technique. This complements a different method for deleting rat genes described last year in *Science*. In addition to having two ways to knock out target genes, other groups have reported advances in techniques to produce transgenic rats as well as random knockouts that could be screened for interesting mutants. “It is an exciting time for rat genetics,” says Aitman.

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unlike mice, are sociable and easily trained. Rats, being more intelligent than mice, are also expected to be better models of diseases such as Alzheimer’s and Parkinson’s. “And rats are bigger, so it’s much easier to get blood and other samples for analysis and to perform other mechanistic studies,” says Xiaohui Wu, a geneticist at Fudan University in Shanghai.

Researchers have developed other rat knockout techniques. One relies on ENU, a chemical agent that triggers mutations when injected into animals. Another uses transposons, DNA segments that jump from one spot to another within a genome, occasionally disrupting a gene. But both approaches cause random mutations, which means researchers cannot target specific genes.

Then last year, Aron Geurts, a molecular geneticist at the Medical College of Wisconsin in Milwaukee, and his colleagues surprised the field by reporting that they had adapted to rats a method used to knock out

U.S. SCIENCE BUDGET

Senator Builds His Legacy With University of Alabama Earmarks

The recession has put a huge crimp in plans by many U.S. universities to expand their research capacity, with Harvard University's stagnant billion-dollar Allston campus a prime example (*Science*, 27 February 2009, p. 1157). But a sluggish economy has been no obstacle for the flagship campus of the University of Alabama, which is speeding ahead with a four-building, \$275 million science and engineering corridor.

What's Alabama's secret? Senator Richard Shelby (R-AL).

Using his position as a senior member of the Senate Appropriations Committee, Shelby has single-handedly implemented an ersatz federal academic infrastructure program for the Tuscaloosa campus over the past decade. Last month, Shelby won the committee's approval for a final round of financing for the project, due to be completed in 2014, with \$30 million from the \$48 million construction budget of the National Institute of Standards and Technology and another \$15 million from the budget of a health services agency within the Department of Health and Human Services. If approved in the final 2011 spending bill, Shelby's earmarks for the project will total \$135 million since 2008. The number jumps to \$170 million with the inclusion of an earlier round of funding for the first of the four buildings, the \$60 million, eponymously named Shelby Hall that opened in 2004.

Academic earmarks have been around since the 1980s (*Science*, 25 July 2008, p. 480). Although most science policymakers believe earmarks weaken U.S. science by placing the preferences of individual lawmakers above those of the peer-review process, politicians have used them to benefit thousands of institutions in their home states.

Earmarking is a sensitive topic for many universities, which support peer review but like federal funding, too. "We recognize that our institutions work with members to obtain earmarks, based on their ability to make a compelling case for their role in fostering economic development and building research capacity in the state," says Jennifer Poulakidas, head of congressional and governmental affairs for the 218-member Association of Public and Land-Grant Universities in Washington, D.C., of which the three-campus Alabama system is a member.

Shelby's efforts, observers say, represent an unprecedented bounty for a single school at a time of growing opposition to the practice. This year, for example, Democrats in the House of Representatives have agreed not to propose any earmarks for companies and other for-profit institutions, and House Republicans have decided to eschew all earmarks. Their Senate colleagues have made no such promises, however.

Running in November for his fifth 6-year term, Shelby says the earmarks he has secured for several of the state's universities have been good for Alabama's economy and its scientists. "The federal funding [I] have been able to help procure for math, science, and engineering facilities at Alabama universities has directly impacted the State's ability to compete on a national level," he said in a statement to *Science*. "Before [I] began working to bring state-of-the-art math, science, and engineering facilities to universities across Alabama, only a few schools nationwide were able to compete for federal research funding. Now, Alabama has some of the best research facilities in the nation and regularly competes for these dollars."

Joe Benson, Alabama's vice president for research, says the corridor project has allowed the university, which in 2008 ranked 203rd among U.S. schools in the amount of research funding it receives from all sources, to improve the caliber of its faculty. "The facilities allow us to attract better faculty, and it makes them more productive once they're here," Benson explains. That productivity stems in part from better graduate students; Benson says test scores for incoming students have risen 15% since 2003. The university is also pouring money into dozens of new faculty positions, especially in those departments, including chemistry and engineering, that occupy the two buildings already completed—the second opened last fall. "There are many institutions that are not hiring, or letting people go, while we've been able to grow," he notes. "We've been very fortunate."

Benson hopes Alabama can increase its overall research expenditures by 50% over the



Heavy lifting. Senator Richard Shelby has provided \$170 million for four science buildings on the Tuscaloosa campus.

next 5 years and move into the top 150 schools nationally. Shelby's help has made such a goal feasible, he adds.

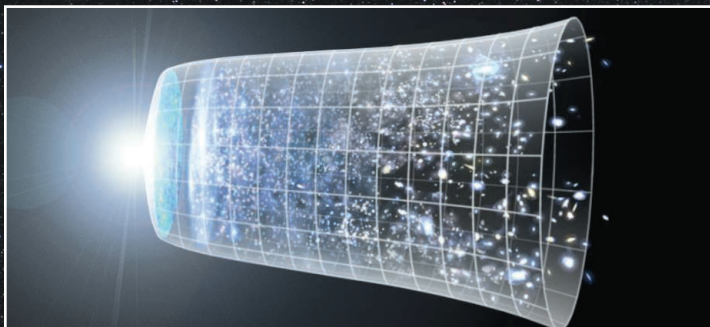
"Without the earmarks, we would have continued to invest in our infrastructure. But we wouldn't be nearly as far down the road as we are now," says Benson. The earmarks represent slightly more than half the total cost of the four buildings, says Benson, with the rest divided nearly evenly between state-financed bonds and the university's own funds.

Higher education officials say that earmarks like Shelby's demonstrate the need for a competitive academic infrastructure program within the federal government that would fund the most worthy campus projects. "A federal program would have a tendency to reduce requests for academic earmarks," says Barry Toiv of the 62-member Association of American Universities in Washington, D.C., one of several groups plumping for the concept. But a new report by Citizens for Responsibility and Ethics in Washington, which opposes earmarks, suggests that the practice may be ingrained in the political system. The nonprofit documents how Shelby has funneled \$267 million since 2008 to clients of organizations employing several former staffers-turned-lobbyists. It also notes that, in the past decade, those lobbyists and their clients have given nearly \$1 million to Shelby's political action committees.

"It's become part of what's expected, for ex-staffers to hang out a shingle and then lobby their former bosses," says Steve Ellis of Taxpayers for Common Sense in Washington, D.C., another federal fiscal watchdog group. "They are such an easy target." Ellis pauses, then adds, "But I have to admit that it's a pretty staggering number that Shelby has been able to direct to the Crimson Tide."

—JEFFREY MERVIS

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Wide-Field Infrared Telescope (builds on Joint Dark Energy Mission)

Goal: Investigating dark energy, searching for exoplanets

Total cost: \$1.6 billion

Cost to U.S. government (2012–2021): \$1.6 million

Launch date: 2020

Augmenting the Explorer program

Goal: Small to midsize missions that target specific topics such as brown dwarfs and gamma-ray bursts

Total cost:
\$463 million

Cost to U.S. government (2012–2021): \$463 million

Launch date: Rolling



CREDITS (LEFT TO RIGHT): NASA, JAXA

ASTRONOMY

U.S. Astronomers Unveil Stripped-Down 'Short List'

In 2008, when a committee of U.S. astronomers led by Stanford University astrophysicist Roger Blandford was asked to recommend funding priorities in astronomy and astrophysics for the next decade, it was clear what the panel was not supposed to do. "The message from Congress was: Don't give us a list of 50 things to fund," says panelist Debra Elmegreen, an astronomer at Vassar College in Poughkeepsie, New York. "Give us the things you really, really want to do."

That's exactly what the panel says it has done in its report of the sixth decadal survey, released last week by the National Research Council. Unlike previous decadal surveys, which often produced unrealistically long "wish lists" of priorities, the new report claims to have made some hard choices that hew to the realities of a tough

budgetary climate. And for the first time, the survey had estimates of project costs vetted independently, which the panelists say makes those figures more realistic than in the past.

The report identified four major projects each in the space- and ground-based categories (see graphic). The top choices in both groups concern dark energy, the mysterious force that is accelerating the expansion of the universe. On land, the panel chose the \$463 million Large Synoptic Survey Telescope (LSST), an 8.4-meter optical telescope that will help investigate dark energy, supernovae, and other areas. In space, the top choice was the \$1.6 billion Wide-Field Infrared Survey Telescope (WFIRST)—until now known as the Joint Dark Energy Mission—which should enable researchers to study dark energy, find

Earth-like planets, and survey galaxies, including our own.

"It's great that the committee saw the excitement and possibility of studying dark energy," says Adam Riess, an astrophysicist at Johns Hopkins University in Baltimore, Maryland. Riess says he is especially pleased with the endorsement for WFIRST, which he calls a "crucial capability in space that a number of disparate investigations need for their science."

Both projects have been in the works for a few years and have received public and private funds for planning and design. In fact, LSST was among three major ground-based initiatives recommended for support in the 2001 decadal survey. Led by a consortium of institutions headed by astronomer J. Anthony Tyson of the University of California,

Large Synoptic Survey Telescope

Goal: Studying dark energy, dark matter, supernovas, near-Earth objects

Total construction cost: \$465 million

Cost to U.S. government (2012–2021): \$421 million

Annual operating cost: \$42 million

Science begins: Late 2010s



Mid-Scale Innovation Program

Goal: Funding projects that cost between \$4 million and \$135 million, such as a new radio array to study the sun

Total cost: \$93 million to \$200 million

Science begins: Mid-to-late 2010s



CREDITS (LEFT TO RIGHT): T. MASON, MASON PRODUCTIONS INC. / LSST CORP.; FASR

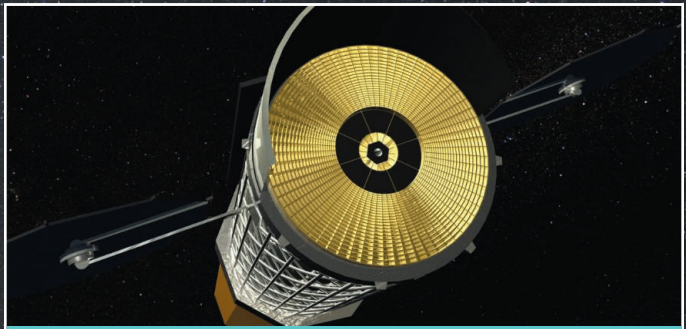
Laser Interferometer Space Antenna (LISA)

Goal: Detecting gravitational waves and black-hole mergers; testing general relativity

Total cost: \$2.4 billion

Cost to U.S. government (2012–2021): \$852 million

Launch date: 2025



International X-ray Observatory (IXO)

Goal: Studying black-hole accretion and neutron-star physics; stellar astrophysics

Total cost: \$5.0 billion

Cost to U.S. government (2012–2021): \$200 million

Launch date: 2020s

SPACE-BASED PROJECTS

Davis, the project has already picked out a site in Chile and finished casting its primary mirror.

"We are recommending that the National Science Foundation enter LSST into its MREFC line as soon as possible," says Blandford, referring to the account through which NSF funds the construction of major research facilities. NSF officials seem receptive to that message. "We're very excited by having LSST as number one among ground-based projects," says Jim Ulvestad, NSF's director for astronomical sciences.

WFIRST's fate appears to be less certain, partly because delays in the \$4.5 billion James Webb Space Telescope, scheduled for launch in 2014, could curtail NASA's ability to fund new missions. However, the project that WFIRST builds on has the support of both NASA and the Department of Energy. The two agencies are in talks with the European Space Agency about a possible partnership, which would boost WFIRST's prospects of being launched by the panel's recommended

date of 2020. "The U.S. should play a leading role in such a partnership," Blandford says.

Blandford says the panel considered two budget scenarios: one in which U.S. funding for the physical sciences doubles over the next decade, and one that sees only modest increases. All of the projects recommended in the report could be implemented under a doubling. In the less-rosy scenario, Blandford says, a number of existing observatories—particularly ground-based ones—should be shut down to make room for the new initiatives.

Of course, the viability of the panel's recommendations hinges on the accuracy of cost estimates of the different projects. Previous surveys have drawn fire for providing estimates as low as a fifth of the ultimate costs for some missions. This report's estimates are more believable, Blandford says, because they were evaluated by an independent contractor, Aerospace Corp., instead of by other astronomers as in previous surveys. "Astronomers are not really good business man-

agers," says panelist Marcia Rieke, an astronomer at the University of Arizona, Tucson. In this survey, some of the estimates submitted to the panel "drew gasps from the independent evaluators," she says. "They looked at some of the concepts and said it was impossible to cost them because so much engineering needed to be done to even begin to estimate the project cost."

Blandford says the panel also tried to strike a balance between large and small projects. "We strove to protect the smaller and nimbler activities," he says. That's why ranked second in the space category is a proposed augmentation to the Explorer program, which supports small- and medium-sized missions with specific science goals. Similarly, in the category of large-scale, ground-based projects, right behind LSST is a recommendation to fund a Mid-Scale Innovations Program within NSF to fund projects that cost more than \$4 million and less than \$135 million. **—YUDHIJIT BHATTACHARJEE**

GROUND-BASED PROJECTS

Thirty Meter Telescope



Giant Segmented Mirror Telescope

Goal: Studying the earliest galaxies and galaxy evolution; detecting and characterizing planetary systems

Total construction cost: \$1.1 billion to \$1.4 billion

Cost to U.S. government (2012–2021): \$257 million to \$350 million

Annual operating cost: \$36 million to \$55 million

Science begins: Mid-2020s

Giant Magellan Telescope



Atmospheric Čerenkov Telescope Array

Goal: Detecting dark matter, investigating active galactic nuclei

Total construction cost: \$400 million

Cost to U.S. government: \$100 million

Operating cost: Unknown

Science begins: Early 2020s

Free Journals Grow Amid Ongoing Debate

Ten years ago, a few scientists started an 'open access' campaign for free journals funded by author fees. Their flagship, the Public Library of Science, is expected to break even soon—but remains controversial

A DECADE AGO, THREE U.S. BIOMEDICAL scientists vowed to start a revolution in science publishing. They wanted to persuade publishers to share research papers normally available only to paying customers in a free online library. The trio threw their weight behind a radical idea: charge authors a fee, give them copyright, and post their peer-reviewed papers on the Internet immediately for anyone to read.

The scientists called their venture the Public Library of Science (PLOS), echoing a frustration among librarians over the escalating cost of journals. They argued that taxpayers shouldn't have to buy subscriptions to see the results of research they had already paid for. Making the world's research papers freely available would "vastly increase the accessibility and utility of the scientific literature, enhance scientific productivity," and bring together disparate communities in biomedicine, wrote PLOS's founders, including

Harold Varmus, the former director of the National Institutes of Health (NIH) who now heads the National Cancer Institute.

Today, the so-called open-access movement is claiming success. Publishers big and small are producing hundreds of free-to-read, peer-reviewed online journals that charge authors fees ranging from about \$500 to \$3000 per paper. (By various measures, between 7% and 11% of the world's peer-reviewed scientific journals are now open access.) The most prominent publisher, the nonprofit organization PLOS, launched its first journal in 2003. This year, PLOS is on track to make a small profit—a "landmark for PLOS, but also for open-access publishing as a whole," testified Catherine Nancarrow, a managing editor of PLOS, at a U.S. congressional hearing last month.

Many biomedical scientists are required by their funding agencies to practice a limited kind of open access by sending manuscripts

of their published papers to free archives like NIH's PubMed Central. A recent study finds that 20% of peer-reviewed articles across all disciplines are now freely available mainly through journals or as manuscripts in online repositories (see graph, p. 898). (This includes journals that make them available after a delay; *Science* does so 1 year after publication.) The portion freely available is growing by about 1% a year, says study leader Bo-Christer Björk of the Hanken School of Economics in Helsinki.

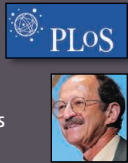
Although the gains seem modest, "that is substantially further than anyone would have thought we would have gotten," says computational and evolutionary biologist Michael Eisen of the University of California, Berkeley, one of PLOS's founders and a board member. The open-access movement "has been remarkably successful, and the momentum now is in our direction."

U.S. policymakers are considering whether to expand NIH's paper-sharing policy to other research agencies. This proposal—and the broader open-access campaign—remains rife with controversy, however. Debates rage about whether open access is speeding scientific progress. Some argue that academic researchers already have good access to the articles they need. Critics suggest that the open-access publishing model encourages mediocre work, noting that PLOS, for example, has succeeded financially only because one of its journals collects fees on thousands of lightly reviewed papers a year.

Some traditional publishers—including many scientific societies—fear that at some tipping point in the future, libraries will drop subscriptions and put journals out of business. But so far, the journals haven't shown that public-access mandates have done them harm.

Stick and carrot

Although physicists have shared manuscripts publicly online for 2 decades, the practice was rare in biomedicine until 2000. That year saw the debut of London-based open-access biomedical publisher BioMed Central, founded by entrepreneur Vitek Tracz. The company initially charged authors no fees and planned

2000	2002	2003	2005
- Public Library of Science (PLOS) founded - NIH launches PubMed Central - BioMed Central open-access publisher debuts 	- BioMed Central begins charging \$500 author fee - Howard Hughes Medical Institute (HHMI) agrees to pay author fees 	PLOS launches <i>PLOS Biology</i>, author's fee \$1500 	NIH, Wellcome Trust ask grantees to post articles in free database after delay  

to recoup costs through other publishing ventures. NIH the same year launched its PubMed Central archive, which Varmus had proposed before moving to Memorial Sloan-Kettering Cancer Center in New York City. It was also in 2000 that Varmus, Eisen, and Stanford University geneticist Patrick Brown founded PLoS. They collected more than 30,000 researchers' signatures on an open letter threatening to boycott journals that didn't allow their papers to be shared freely on PubMed Central within 6 months of publication.

The threat didn't have a big impact, but PLoS's leaders found a better way to foment revolution. With a \$9 million grant from the Gordon and Betty Moore Foundation, PLoS launched a free journal, *PLoS Biology*, in 2003. The new money allowed it to hire talent from top journals such as *Cell*. The nonprofit burned through its grant rapidly at first, and PLoS sharply raised its initial author fee of \$1500. (Today, fees at its six subject-specific journals range from \$2250 to \$2900; hard-pressed authors can ask for a fee waiver.) In 2006, PLoS's fortunes improved after it launched the multidisciplinary *PLoS ONE*, which featured a new peer-review model: Reviewers would check articles for scientific rigor but not for importance, and authors of accepted papers would pay a fee of \$1350.

Submissions to *PLoS ONE* have soared—along with PLoS's revenue. It expects to publish about 7500 papers this year, making it the world's largest journal in terms of volume, PLoS says.

BioMed Central, which began charging author fees in 2002, now charges about \$1300 to \$2400 per paper in most of its 206 journals. It "has been profitable for some time," says Managing Director Matthew Cockerill. As evidence, he points out that BioMed Central was snapped up in 2008 by Springer, which, like other giant commercial publishers, is starting its own open-access journals. Another success is the bargain-rate Hindawi, based in Cairo, which



Rocking the boat. PLoS founders Brown, Eisen, and Varmus.

puts out more than 200 open-access journals in biomedicine and other fields, charging \$600 to \$1500 per paper. Some open-access journals published by societies, such as the *New Journal of Physics* and *Optics Express*, are at the top of their field in impact factor (a measure of how often a journal is cited). "They're working because the community got behind them," says Mary Waltham, a publishing consultant in Princeton, New Jersey.

An academic project called the Directory of Open Access Journals now tracks some 5000 scholarly and scientific journals (up from 861 in 2003). Only two-thirds are peer reviewed, and a couple of publishers, says Björk, "seem more interested in collecting author fees than assuring quality." Still, the list includes respected journals, including many in developing countries that charge no author fees. Ulrich's Periodicals Directory counts 2888 of 27,252 peer-reviewed academic journals as open access, or 10.6%, notes Björk. Marie McVeigh of Thomson Reuters, which derives impact factors for high-quality science and social science journals, says 622 of these 9190 journals are open access, or 6.8%.

The field has received a boost in recent years from so-called public-access policies at funding agencies. NIH, for example, has required grantees since April 2008 to submit copies of their accepted peer-reviewed manuscripts to PubMed Central for posting within 12 months. NIH's chief digital library,

David Lipman, testified last month at a U.S. House hearing that business is booming. The hearing had been called to consider a bill to extend the NIH policy to 11 more agencies and shorten to 6 months the permitted delay in releasing manuscripts. Lipman says PubMed Central attracts 420,000 visitors each weekday. Only 25% of them are using university computers, which suggests that the archive "has become a broad-based repository" for patients, students, and clinicians as well as researchers, Lipman says.

Several other U.S. and European funding agencies, such as the Howard Hughes Medical Institute (HHMI) and Wellcome Trust, have adopted similar mandates for their grantees. Both also offer to pay authors' fees for publishing in open-access journals.

Universities are keen on open access as well. A growing number of institutions, such as Harvard University's School of Arts and Sciences, now ask faculty members to deposit manuscripts in institutional repositories. And many are setting up funds to help pay author fees. Harvard's Stuart Shieber says the goal is to make it easier for publishers to convert journals to open access.

Quantity subsidizes quality

Detractors have criticized *PLoS ONE*, which publishes 69% of submissions, for making money by publishing marginal research. They point out that PLoS's highly ranked journals *PLoS Biology* and *PLoS Medicine*, which reject a much higher portion of submissions, aren't sustainable without a subsidy. The more selective the journal (and therefore higher ranked), the more it costs to produce. That's because it's expensive to manage a rigorous peer-review system, and each rejection represents a lost author fee. High-impact journals *Science* and *Nature*, which also publish news and nonresearch sections, say they have per-article costs of \$10,000 or more—financed in part by subscriptions and advertising.

But supporters of PLoS defend its business model. "There's no shame in the fact that

Online

sciencemag.org

Podcast interview
with author
Jocelyn Kaiser.

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2006

- Bill expanding NIH policy to more U.S. agencies
- U.K. Medical Research Council mandates free access to articles within 6 months
- PLoS raises highest author fee to \$2500, launches *PLoS ONE*

2007

HHMI

- HHMI mandates free access to articles within 6 months
- U.K. PubMed Central debuts

2008

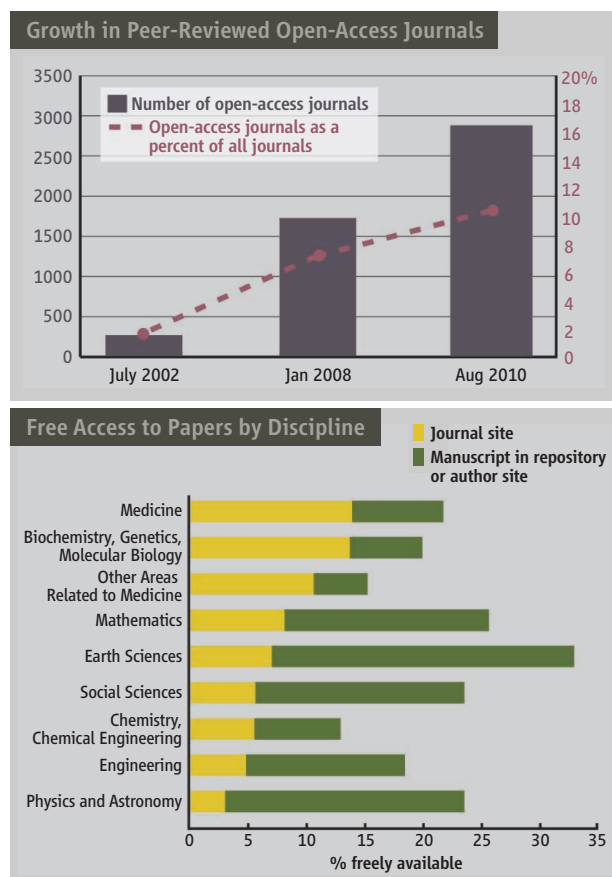
- BioMed Central sold to Springer
- NIH public-access policy becomes mandatory
- Harvard Arts and Sciences faculty agree to put papers in free university repository

2009

- Harvard and four other universities commit to create funds to cover author fees

2010

- Roundtable report endorses public access across U.S. research agencies
- PLoS expected to break even



Free for all. Open-access journals are on the rise; about 20% of a sample of 1837 articles published in 2008 were eventually free.

PLoS ONE is fueling a profitable business,” says Eisen. And he says it has good papers. *PLoS ONE* recently received an initial impact factor that put it in the top 25% of biology journals. Even a skeptic of open access, Martin Frank, executive director of the American Physiological Society in Bethesda, Maryland, says, “They did damn well.” Like others, though, he is puzzled by the results and suggests that they may have been skewed by a few blockbuster papers. Open-access publishers are sensitive about the quality issue. Cockerill notes that one aim of the Open Access Scholarly Publishers Association founded in 2008 is to establish standards and distinguish “reputable journals” from the pack.

Another key dispute centers on the claim that open access gives scientific results wider circulation and use. Several scholars have found that open-access papers are cited at least 100% more often than papers available only by subscription—suggesting that they are more widely read. But critics say these studies failed to control for a bias: Editors and authors tend to make only their most important papers available for free.

A study last year found only an 8% citation advantage for open-access articles, although

the rate was higher in developing countries (*Science*, 20 February 2009, p. 1025). Philip Davis, a graduate student in science communications at Cornell University, has done what he says is the only randomized controlled trial to examine the issue. For his unpublished dissertation, he worked with seven publishers of 36 journals (including *Science*), mostly in biomedicine and social sciences. The journals randomly made 712 of 3245 papers open access. Davis found that after 2 years the open-access papers weren’t cited any more often or more quickly. “For the research community, access is essentially a nonissue,” Davis concludes.

Davis found another story when he looked at usage: Open-access papers were downloaded twice as often as others in the first year. This result suggests that the public might be benefiting from open access, Davis says.

But Eisen says such evidence isn’t crucial: “It is a very hard thing to quantify. You sort of have to accept that it’s a good thing on first principle to have papers freely available.”

Free but expensive?

The move to expand open-access mandates doesn’t please traditional publishers. Some fear it will eventually kill subscription journals. Allan Adler, vice president for legal and governmental affairs for the Association of American Publishers, points to a 2006 survey of librarians finding that if two-fifths of a journal’s articles became free within 12 months, 44% said they would cancel their subscriptions. A research study cofunded by the European Union involving 12 major publishers and 300 journals is studying potential impacts; results are expected next year. It’s too soon to know what the impact of NIH’s mandate will be, says Frank.

Other experts see a strong economic rationale for open-access publishing. A model developed by John Houghton and colleagues at Victoria University in Melbourne, Australia, assumes that author-pays journals save costs and that wider access to papers helps industry scientists in particular. Houghton’s

group has projected that open-access publishing could save three European countries hundreds of millions of euros a year. In preliminary work, he finds that the proposal to extend NIH’s policy to more U.S. agencies would yield benefits to the U.S. economy of more than \$1 billion over 30 years—five times the cost. Because research investments have a high rate of return, says Houghton, even a 1% gain in access “can result in a substantial cost saving.” Publishers, meanwhile, have attacked Houghton’s model as relying on flawed assumptions.

Even advocates of the revolution admit they’re not sure how publishing costs would be distributed in a world totally converted to open access. Questions loom about who will pay. Harvard’s Shieber says that funding agencies can make up for what they spend on grantees’ author fees by reducing the overhead money they add to NIH grants, because universities won’t need to spend as much for library journal subscriptions. “It’s all the same money,” he says. But Frank is skeptical that universities would tolerate a reduction in overhead rates. If the cost is charged to NIH grantees, Frank warns, that will leave less money for doing research: “It’s going to come out of research dollars.”

Despite the hoopla and contention, perhaps the chief obstacle to making more papers freely available is that the average scientist just isn’t engaged. Two years after the NIH policy became mandatory, only 70% of eligible manuscripts are being deposited, and only 40% at the Wellcome Trust. Many “hybrid” journals that offer authors the option of paying a fee (as high as \$5000 for *Cell* and *Nature Communications*) for immediate free access say uptake is less than 10%.

“It’s going to depend on the scientist,” says Avice Meehan, communications chief for HHMI in Chevy Chase, Maryland. For some, open access is of “paramount importance,” she says, but “for others, their priorities lie elsewhere.”

The future of open access likely will depend on what funding agencies do—and particularly on the subsidies they provide. Tighter budgets will add to libraries’ demands for more open-access journals, says industry analyst Claudio Aspesi of Sanford Bernstein. But tighter budgets could also limit public support for author fees. For the time being, Aspesi and many others expect that traditional and open-access journals will coexist. “It will be a mixed economy,” says Waltham. “I don’t think it’s ever going to take over entirely. And that’s healthy overall.”

—JOCELYN KAISER

SOURCES (TOP): M. LAAKSO AND B. BJÖRK/HANKEN SCHOOL OF ECONOMICS, BASED ON TENOPIR (2002); T. HEDLUND ET AL. (2004); B. BJÖRK ET AL. (2009) AND ULRICHWEB QUERY (5 AUG 2010); (BOTTOM) B. BJÖRK ET AL., PLOS ONE 5

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On the block. Founded by N. I. Vavilov in 1926, the Pavlovsk station could be dismantled soon.



PLANT SCIENCE

Pavlovsk's Hopes Hang on a Tweet

A Russian research center with a unique collection of European fruits and berries could be bulldozed for housing unless a legal decision is reversed

MOSCOW—Scientists trying to save a unique gene bank of thousands of fruit and berry crops in Russia have taken heart from a surprise intervention last week by Russian President Dmitry Medvedev. He sent out a “tweet” from his Twitter account saying that he wants to review a court decision that threatens to destroy the collection. Medvedev’s note came amid an international campaign to rescue the Pavlovsk Experimental Station of the N. I. Vavilov Institute of Plant Industry near St. Petersburg, whose lands have been approved for bulldozing by property developers.

More than 6000 varieties of apples, plums, strawberries, and other fruits could be obliterated in what scientists say would be a significant blow to global food security. The station is the largest repository in the world of European fruits and berries. Its value lies in the immense diversity of its collection and the opportunities it offers plant breeders to screen for beneficial traits that can help crops adapt to climate change, pests, and disease. Up to 90% of its varieties—including more than 1000 strains of strawberries—are not held anywhere else. Many no longer exist in the wild.

The station was dealt a blow on 11 August when Moscow’s arbitration court approved a decision to pass 71 hectares of the station’s state-owned land to the Russian Housing Development Foundation. An appeal concerning a separate 19-hectare plot was rejected in April, and some of the land is expected to go on sale as early as 23 September. But Presi-

dent Medvedev signaled a possible change of fortune 2 days after the arbitration court’s decision, with a tweet that said: “Received the Civic Chamber’s appeal over the Pavlovsk Experimental Station. Gave the instruction for this issue to be scrutinised.” That message appeared to vindicate the tactics of campaigners who had sent hundreds of tweets to Medvedev and lobbied Russian agencies such as the Civic Chamber, a Kremlin-appointed public watchdog.

“This is excellent news,” says Emile Frison of Bioversity International, which has collaborated with Pavlovsk on researching the nutritional benefits of its collection. “I hope President Medvedev will look at it seriously and decide to save this invaluable resource, not only for Russia but for the entire world.” Before the tweet, Pavlovsk station director Fyodor Mikhovich had all but given up hope, telling *Science*, “Our legal options are almost exhausted. ... We need to find some way to stop this madness.”

Pavlovsk Experimental Station was created in 1926 by Nikolai Vavilov, the Soviet botanist and geneticist who is credited with inventing the seed bank. It is a field collection of plants that cannot be stored frozen as seeds. Because they are propagated by grafting, it would take a minimum of 10 years to relocate the collection. “You could say, why not just save the best apple or the best blackcurrant?” says Cary Fowler, executive director of the Global Crop Diversity Trust (GCDDT) in Rome. “But there is no such thing as ‘the best’ in an evolutionary,

biological world. Today’s best variety is tomorrow’s lunch for a new insect.”

GCDDT is working with the Vavilov Institute to translate and digitize its records and hopes to add them to Genesys, a new software system giving access to information from gene banks all over the world. Fowler says not many foreign breeders and researchers have visited the institute to date because of the difficulties of accessing data in Russia. “The upshot of Genesys is that this collection, which is the biggest in Europe, is about to become visible to Europe and everywhere else in the world.”

Fowler adds: “If Pavlovsk is destroyed, it’ll be the biggest 1-day tragedy in my 30-year professional life. We’ve lost a few collections on the way, from neglect, civil strife, war. But this is preventable. A conscious decision is being made by human beings to destroy this diversity. It’ll be gone forever.”

Russian officials have taken little interest in the repository, even as the worst drought in 130 years has wiped out at least a quarter of the country’s grain harvest. “With Europe experiencing unprecedented fluctuations in weather, it brings into sharp focus the need to have a flexible response, to have places like Pavlovsk to underpin crop improvement for the future,” says Mike Ambrose of the John Innes Institute, Britain’s leading center for crop research.

Pablo Eyzaguirre, a senior scientist with the nutrition program of Bioversity International, says the potential health benefits of the Pavlovsk collection are enormous. His organization has been working in partnership with the station and the Gabriel Lippmann Center for Public Research in Belvaux, Luxembourg, to investigate micronutrients in edible honeysuckle and berry varieties.

“They have many northern berries that are tremendously important for functional diets where there isn’t great access to a diversity of fruit and vegetables,” says Eyzaguirre. “So it’s ironic that a country like Russia that is facing a major health crisis from chronic diseases would contemplate destroying such a resource. In fact, it’s barbaric.”

—TOM PARFITT

Tom Parfitt is a Moscow-based writer for *The Guardian*.



LETTERS

edited by Jennifer Sills

Local Programs Take a Bite out of Malaria

THE 14 MAY SPECIAL SECTION ON TUBERCULOSIS AND MALARIA (P. 841) DID NOT SUFFICIENTLY emphasize the development and support of health infrastructure in endemic areas. There is a reason that Zambia is doing well whereas its neighbors have made little progress with malaria. In Zambia, the Ministry of Health is functional and organized. From 1950 until 2000,

Zimbabwe also had a successful malaria control operation, which until recently was staffed and run by local scientists operating in a well-managed health system.

Malaria can be controlled, but to do so we must train local scientists and establish careers for them within these health services. National governments, with the help of the World Health Organization and others, must foster and encourage the establishment of local scientists. Local universities need assistance in developing degree programs to train epidemiologists, biologists, and health managers to take the lead in running and managing the control operations.

To maximize the effect of these programs, we must rapidly collect and process data. Interventions can then be targeted and the disease reduced to smaller and smaller foci. We hope that an organized, locally supported health system can restrict malaria until it is no longer of major public health importance. However, we must remain vigilant: As Zimbabwe has shown, malaria will return with a vengeance if the health system deteriorates.

CLIVE J. SHIFF¹* AND PHIL THUMA²¹Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA.²The Malaria Institute at Macha, Macha Research Trust, Choma, Zambia.

*To whom correspondence should be addressed. E-mail: cshiff@jhsph.edu

National Indicators Show Biodiversity Progress

IN THEIR REPORT "GLOBAL BIODIVERSITY: Indicators of recent declines" (28 May, p. 1164), S. H. M. Butchart and colleagues commented that on a global scale there is little evidence that the rate of biodiversity decline is slowing and some evidence that it is increasing. However, there are some encouraging stories at national levels. For instance, the Chinese government has implemented biodiversity conservation action plans. The proportion of China's gross domestic product invested in environmental pollution control and forestry conservation has increased since 1990 and exceeded 1% since 2001;

the number of nature reserves was 32 times the number in 1978, and the area devoted to reserves was 120 times the area in reserves in 1978 (1).

There is more good news: Some indicators of pressures on biodiversity show declining trends. The total discharge of chemical oxygen demand and the amount of sulfur dioxide in waste gas, two nationally targeted pollutants, decreased 9.66 and 13.14%, respectively, as compared with that of 2005 (2). The discharge of toxic and harmful pollutants in wastewater, the emission intensity of chemical oxygen demand of key industries, the emission of soot dust and industrial dust in waste gases, and the discharge of solid wastes decreased annually

12.24, 21.62, 5.16, and 16.89% since 1998, respectively (1).

Some indicators of the state of biodiversity showed recovering trends. The annual average net primary productivity, including forest resources as well as the area and growing stock of natural forests, has been increasing in the past two decades (1, 3); there is also a steady increase in the Marine Trophic Index from 1997 until now (1). Although substantial progress toward the 2010 target was made (4), we agree that China still faces severe environmental pressures, because total pollutant emissions are still high, and trends toward the loss of habitats, threatened species, and genetic resources are not effectively checked (1).

HAIGEN XU,* HUI DING, JUN WU

Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection of China, Nanjing 210042, China.

*To whom correspondence should be addressed. E-mail: xhg@nies.org

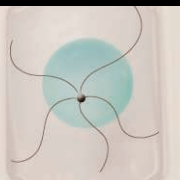
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Response

XU AND COLLEAGUES REPORT SOME ENCOURAGING trends at the national scale for China. This is welcome news and augments the examples we provided (in Table 2) of success and positive trends relevant to the 2010 target. Unfortunately, they do not invalidate the overall picture that humans are destroying wild nature as fast as ever, that much of this loss is irreversible, and that it has ethical implications as well as substantial economic and social costs (1).

The report on China submitted to the Convention on Biological Diversity (CBD) (2) highlights the successes that Xu *et al.* describe but also describes negative trends and



Nuclear
positioning

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Tiny versions of
TV antennas

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BEN COLLEN,³ RICHARD D. GREGORY,⁴
CARMEN REVENGA,⁵ MATT WALPOLE¹

¹United Nations Environment Programme World Conservation Monitoring Centre, 219 Huntingdon Road, Cambridge CB3 0DL, UK. ²BirdLife International, Wellbrook Court, Cambridge CB3 0NA, UK. ³Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK. ⁴Royal Society for the Protection of Birds, The Lodge, Sandy SG19 2DL, UK, and European Bird Census Council. ⁵The Nature Conservancy, 4245 North Fairfax Drive, Arlington, VA 22203, USA.

*To whom correspondence should be addressed. E-mail: stuart.butchart@birdlife.org

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monitoring gaps for other aspects of biodiversity. In particular, pressures on biodiversity in China from factors other than pollution are almost certainly intensifying, and the overall state of biodiversity in China is likely to continue its decline. Furthermore, none of the national reports to the CBD claims that the 2010 target has been met at the country level (3), supporting our conclusion that the target is unlikely to have been met at global scale.

In addition to global indicators, national biodiversity strategies and plans (such as the ones for China that Xu *et al.* discuss), combined with appropriate targets and indicators, will be essential for stimulating, guiding, and monitoring action at the subnational and national scale in order to meet future targets for reducing or halting biodiversity loss.

STUART H. M. BUTCHART,^{1,2*}

JONATHAN E. M. BAILLIE,³ ANNA M. CHENERY,¹

LIFE IN SCIENCE

Up a Creek in Indonesia

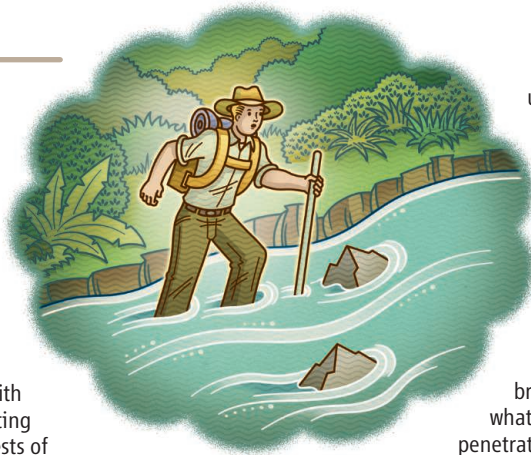
I am jolted awake by someone shouting “banjir!” [flood!]. Flashlight beams sweep across my tent like lightning flashes. I hear the rumble of boulders striking boulders. The river, its water the color of poured concrete, has risen more than 2.5 meters as we slept.

It is 2 a.m. on 2 November 2008. I am in the Kali Ibem river valley in an isolated corner of Indonesian New Guinea. Along with a team of local village naturalists, I am testing a route into the cool and moist upland forests of the Foja Mountains, home to a range of endemic birds, mammals, butterflies, and frogs. We are in the initial phase of Conservation International's second Rapid Biodiversity Assessment of this legendary wilderness. Because it is the rainy season, mist shrouds the upland forests, making foot access to the mountain much more dependable than helicopter. But there is no existing walking track into these pristine mountains.

EDITOR'S NOTE

This is an occasional feature highlighting some of the day-to-day humorous realities that face our readers. Can you top this? Submit your best stories at www.submit2science.org.

In Kwerba village, before this exploratory trek, I had chatted with the village men about this route, and they had agreed that this might be a possible route into the interior. They were just being polite. During this season the Kali Ibem is dangerous to negotiate. After we had waded



up the river for three brutal days, we arrived in territory none of the men had ever visited. I quickly learned why. The river here had cut a deep gorge that becomes treacherous when it rains in the headwaters. Before we were able to get to the ridge that led to the uplands, the river sent us a clear signal: three flash floods in three days.

That alarming night of rising waters threatened but did not flood our camp.

The next day, several hours of hiking upriver brought us to a place even more harrowing than what we had already experienced. The sun barely penetrated the narrow chute. I admitted defeat, and our team turned back.

With bruised feet swollen from slogging for days in water, we dragged ourselves back to Kwerba village. Determined to complete our mission, we used our satellite phone to call for a helicopter, which transported the field team to the high country.

We escaped harm by the skin of our teeth. But in doing so we were able to visit a place that had not seen humans for at least a generation. At dawn and dusk, the river edge teemed with wildlife—we had marvelous close encounters with stolid and staring northern cassowaries, beach-foraging lowland wallabies, placid grizzled tree-kangaroos, unwary Victoria crowned pigeons, even speedy monitor lizards. I had never before had such great encounters with big game in New Guinea, where the wildlife is famously shy. The Kali Ibem was a naturalist's Eden.

BRUCE M. BEEHLER

Science and Knowledge Division, Conservation International, Arlington, VA 22202, USA. E-mail: bbeehler@conservation.org

developing nations. Census data is primarily used to direct national and international policy priorities and is used prolifically in across-nation studies by social scientists and economists [e.g., (1, 2)].

Despite growing awareness of the need to understand the capacity of different scales of governance to adapt to social, economic,

and ecological change (3), census data is rarely used at more local governance scales (4), where change more directly affects the lives of the constituents. Increased collaboration between academia and national statistics offices in developing countries presents an immense opportunity to improve the well-being of the world's poor and to understand

across-scale social and economic dynamics. Key to enabling this opportunity is greater openness and transparency from national governments, a greater willingness of academia to work on challenges in developing nations, and increased focus on such collaboration by funding bodies.

TOM DAVID BREWER

ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, 4811, Australia. E-mail: tom.brewer@jcu.edu.au

TECHNICAL COMMENT ABSTRACTS

Comment on "The Silicate-Mediated Formose Reaction: Bottom-Up Synthesis of Sugar Silicates"

Hyo-Joong Kim and Steven A. Benner

Lambert *et al.* (Reports, 19 February 2010, p. 984) reported that silicate ions catalyze the formation and stabilization of four- and six-carbon sugars from simple sugars, suggesting a possible prebiotic pathway for the synthesis of biologically important sugars. Here, we show that silicate has minimal impact in these respects, especially when compared to borate minerals.

Full text at www.sciencemag.org/cgi/content/full/329/5994/902-a

Response to Comment on "The Silicate-Mediated Formose Reaction: Bottom-Up Synthesis of Sugar Silicates"

Joseph B. Lambert, Senthil Andavan Gurusamy-Thangavelu, Kuangbiao Ma

We reported that silicate mediates the formation of four-carbon and six-carbon sugars from simple two- and three-carbon molecules. Kim and Benner's contention that silicate has little impact on these reactions is based on experiments using harsher conditions of temperature, pH, silicate concentration, and reaction time than in our study, thus confounding any comparisons between our results.

Full text at www.sciencemag.org/cgi/content/full/329/5994/902-b

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.



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Comment on "The Silicate-Mediated Formose Reaction: Bottom-Up Synthesis of Sugar Silicates"

Hyo-Joong Kim and Steven A. Benner*

Lambert *et al.* (Reports, 19 February 2010, p. 984) reported that silicate ions catalyze the formation and stabilization of four- and six-carbon sugars from simple sugars, suggesting a possible prebiotic pathway for the synthesis of biologically important sugars. Here, we show that silicate has minimal impact in these respects, especially when compared to borate minerals.

Lambert *et al.* (1) recently suggested that aqueous sodium silicate mediates aldol reactions between simple C1 to C3 sugars (formaldehyde, glycolaldehyde, and glyceraldehyde) to produce tetroses (C4 sugars) (erythrose and threose) and pentoses (C5 sugars: arabinose, xylose, lyxose, and ribose) and other higher sugars. To support their suggestion, they generated product mixtures from these precursor carbohydrates, both with and without silicate. They then used the differences in the mass spectra of those mixtures to conclude that silicate influenced the relative amounts and stabilities of carbohydrate products formed. From this, they concluded that silicate can guide aldol reactions between these carbohydrates and that this guidance might have helped generate carbohydrates prebiotically on early Earth, where mineral silicate was undoubtedly present.

As part of work examining the role of minerals containing borate in guiding similar prebiotic chemistry (2), we performed essentially the same experiments some time ago. Our work was motivated in part by the knowledge that both silicate and aluminate are more abundant on Earth than borate (3), a 2004 report by Lambert *et al.* containing mass spectral evidence that certain cyclic carbohydrates bind silicate (4), and a 2003 report from Kinrade *et al.* showing that silicate binds ribose (5).

As analytical methods, we used ^{13}C -labeled starting materials and measured the loss of their ^{13}C resonances relative to ^{13}C signals from standards within the sample. As discussed further below, these analytical methods are different from, but we contend superior to, those used by Lambert *et al.* (1). For further analysis, we isolated acetyl and acetone derivatives of the products. Products containing C=O groups were also quantitated by

high-performance liquid chromatography of their dinitrophenylhydrazone derivatives, using ultraviolet spectroscopy to determine their relative amounts ($\pm 5\%$). All of our reactions are run in buffer with controlled pH, because the rates of most reactions involving carbohydrates are sensitive to pH.

Because Lambert *et al.* (1) did not mention a pH for their reactions, we cannot be certain that the conditions that we used to explore the impact of silicate on aldol processes were exactly the same as theirs. However, we, like Lambert *et al.* (1), incubated glyceraldehyde (our starting material had a $3\text{-}^{13}\text{C}$ label) with glycolaldehyde in the presence of silicate (220 mM, pH 11.8 ± 0.1). Further, we studied the stabilities of carbohydrates in the presence of silicate (and other mineral anions) over a wide range of conditions. We appeared to have used the same concentrations of silicate as they used, and tested both longer and shorter times, so that we could be certain that we did not miss anything relevant to the conclusions of interest to the prebiotic community.

Our results are incompatible with the results reported in (1). At both 65°C and room temperature, we found that the major product arising from reaction of $3\text{-}^{13}\text{C}$ -glyceraldehyde with glycolaldehyde in the presence of silicate (220 mM) is arabinose, after 1 hour (at the higher temperature) and 4 days (at the lower temperature). A role for silicate in guiding arabinose formation is incompatible with these results, because they can be explained entirely by the fact that arabinose is simply the most stable of the four pentoses at pH 11.8. The formation of other pentoses, including ribose, in borate buffer is already documented (2); the observation that 2-hydroxymethylerythrose is formed in the presence of borate (but not silicate) is a key item of evidence showing that borate actually does guide aldol reactions but that silicate does not; 2-hydroxymethylerythrose may be an intermediate in the borate-moderated synthesis of pentoses.

We also asked whether silicate could stabilize preformed carbohydrates against decomposition

in base. Again, we observed at most only a very small impact. For example, upon incubation of $5\text{-}^{13}\text{C}$ -ribose in silicate, carbonate, and borate buffers (all 200 mM at pH 11.8), after 1 hour at 65°C , 40% of the ribose stabilized by silicate remained, whereas 24% of the ribose stabilized by carbonate remained. Essentially all of the ribose stabilized by borate remained in these experiments. Separate controls suggested that carbonate does not stabilize ribose against alkaline decomposition. This allows carbonate to be used as a buffer to reproduce Lambert *et al.*'s negative control, rather than measuring product ratios of carbonyl compounds in an unbuffered reaction. Thus, the differences in our results cannot be attributed to differences in procedures. Instead, they suggest that any stabilization of ribose by silicate is small. Similar experiments with silicate, arabinose, and 2-hydroxymethylerythrose produced similar results.

How do we account for the contradictions between our observations and those of Lambert *et al.* (1)? First, it appears that their control was a reaction run in unbuffered aqueous NaOH at the same pH as their reactions with silicate. As the rates of aldol reactions are quite sensitive to pH, neither rate constants nor product estimates for such reactions should be obtained in unbuffered media. Their reported impact of silicate on stability ("less than half") might be consistent with our results had there been an excursion in pH by 0.2 units in their control.

We also question Lambert *et al.*'s use of electrospray mass spectrometry to assess the amounts of products in mixtures prepared with and without silicate. Inspection of figure 2 in (1) suggests that the ions being observed are different in the mixtures being compared; the first set of ions have silicate, whereas the second do not. A comparison of the intensities of different ions determined by mass spectrometry across two different ion systems cannot provide estimates of the actual ratios of products in the two product mixtures. Therefore, we disagree with Lambert *et al.* (1) that silicate mediates formose-like aldol reactions. Further, even if silicate does assist in stabilization of carbohydrates in alkaline solution, the assistance appears to be small compared with that offered by borate.

More positively, borate might be viewed as stabilizing carbohydrates too much, preventing them from reacting further when further reaction is desired. Silicate, through its weaker interaction, does not have this disadvantage. As the central issue in the prebiotic chemistry of carbohydrates arises from the need to have reactions occur when they are wanted (to form higher carbohydrates or convert them to nucleosides, for example) without having undesired reactions occur at the same time, Lambert *et al.*'s observations are important. Other mineral ions, including molybdate, might also help manage the need for some (but not too much) reactivity in prebiotic carbohydrate synthesis, as molybdate interconverts branched and unbranched

Foundation for Applied Molecular Evolution, The Westheimer Institute for Science and Technology, 720 SW 2nd Avenue, Suite 201, Gainesville, FL 32601, USA.

*To whom correspondence should be addressed. E-mail: sbenner@ffame.org

carbohydrates without the need for intermediate aldol fragmentation.

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ECONOMICS

Sustainability and Sources of Wealth

Bobbi S. Low

Paul Collier is a key figure among policy-makers and academics concerned with large-scale sustainability. He stands somewhere in the middle of (and perhaps a little orthogonal to) the spectrum bookended by William Easterly [(1) too simply put: “fix what is most fixable first”] and Jeffrey Sachs [(2) too simply: “you must fix multiple things concurrently, or your improvements will not hold”]. This important argument about how to help the worst-off nations in sustainable ways is the central issue of Collier’s *The Plundered Planet*, which follows on his influential *The Bottom Billion* (3). This time the Oxford economist ranges widely to consider specific difficulties in managing human consumption of both renewable (e.g., fish, lobsters) and nonrenewable (oil, tin, diamonds) resources. Along the way he explores such unforeseen consequences as how the developed world’s fascination with using renewable resources (corn and other crops) to produce biofuel caused huge jumps in crop prices, especially in poor countries, and serious destruction of Amazon forests.

Collier begins by considering the ethical positions held by various decision-makers and how those intersect with history, leading to a variety of resource governance arrangements of quite varied effectiveness and efficiency. He extends his consideration of the “natural resources traps” in *The Bottom Billion*. He goes beyond using nature as an asset (mostly nonrenewable resources) and as a factory (mostly renewable) and ends with hope for “restoring natural order.” Although he works at a large scale, the heart of his analysis is, refreshingly, self-interest. When valuable resources are economically defensible, individuals will try to control them and exclude others. This is true for other species as well as humans, but we have additional forces we can call on: third-party arrangements, social contracts, and rule of law. These can allocate resource control, but much depends on the vision and the power of decision-makers. From small

societies to modern nation-states, when monitoring and punishment are difficult, expensive, or impossible, the process devolves to the sort of resource-driven territoriality seen in other mammals; not only is resource control uncertain, but free-riding will be rampant [e.g., (3, 4)]. There are good examples of successful common property regimes (4, 5), though they tend to be rather small and somewhat isolated from global (potentially high-profit) markets.

Solutions to such large and complex problems as Collier tackles are difficult. Collier does better than those who simply exhort us to “have the will” to solve problems. He is convinced that ordinary citizens can solve these large problems—and I hope so. But there is a serious caveat: Such behavior depends on all of us, or some critical mass of us, taking the time and attention to become well informed about the scientific and economic issues. Yet behavioral ecologists and behavioral economists find that cooperation happens principally when certain conditions are met, such as low cost of cooperating and the existence of monetary or social rewards. (Even social rewards work primarily when the group is small and stable and it’s not easy to “take the money and run.”) We discount not only the future but also people we don’t know, other species, and things we don’t perceive easily (when paying attention might cost us something in the short term); we evolved to

do this when we could control little of our environment. So I fear it will be a long slog to get a well-informed and active citizenry.

There seem to be no simple, unitary, universal answers. Add to these basics the fact that the more levels of involved actors and the more heterogeneous those actors (especially in power), the more difficult fostering cooperation for sustainability becomes. Many of us think we can foster cooperation—but it will require some serious interdisciplinary sharing of expertise and

hunkering down to look clearly at some nasty, difficult issues.

I sometimes find Collier’s brush strokes too broad. There are excellent ecological reasons, for example, not to imagine that because lobsters can be sustainably managed, so can carbon emissions. Or to doubt that all common-property regimes will devolve into open-access, plundered resources [e.g., (6–8)]. But such examples merely mean that when reading *The Plundered Planet*, it is best to do a little background reading in unfamiliar areas.

Collier has made an important contribution in bringing together usually separate fields. His efforts may spark scholars in disparate disciplines to cooperate on these important problems—especially scholars who work at different scales (9). That, however, raises a complaint about referencing (which may have been the publisher’s call). “A Note on Sources” includes only 15 titles, offered as background. An actual literature-cited section, with links from specific claims in the text, would have made the book immensely more useful for interested scholars outside the policy field. Alerting the general public (clearly Collier’s aim here) is excellent, and scholars within the author’s field will probably know the specific references. Nonetheless, the crucial problems Collier addresses range across so many levels of complexity, types of actors, and fields of inquiry that attempts to solve them will require effective collaboration among scholars who normally never speak to one another. A detailed list of the underlying references would help jump-start that difficult and seemingly endless process.

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The reviewer is at the School of Natural Resources and Environment, University of Michigan, 440 Church Street, Ann Arbor, MI 48109–1041, USA. E-mail: bobbilow@umich.edu



The Plundered Planet
Why We Must—and How We Can—Manage Nature for Global Prosperity / How to Reconcile Prosperity with Nature

Paul Collier
Oxford University Press, New York, 2010. 287 pp. \$24.95. ISBN 9780195395259. Allen Lane, London. £20. ISBN 9781846142239.

EXHIBITION

Looking Through the Paint

Patricia Fara

As Britain responds to the twin challenges of financial recession and global warming, its museums have been forced to rely on their own resources instead of transporting masterpieces around the world to stage expensive extravaganzas. Like icebergs being rescued from beneath the surface of the ocean, pictures long consigned to basements are being retrieved for mounting on gallery walls. Sometimes, coherence has been sacrificed by displaying a disparate collection of unfamiliar items, but London's National Gallery has ingeniously staged an exhibition unified by a novel theme that enables visitors to see perennial favorites in a new light.

"What can science tell us about who painted a picture and when it was made?" This was the question posed by Ashok Roy, the museum's director of scientific research, in his public lecture on *Close Examination: Fakes, Mistakes and Discoveries*. The intriguing show explores the crucial contributions made by scientists when identifying works of art and provides an unusual opportunity to witness the forensic detective work taking place behind restorers' closed doors. Co-curator Marjorie Wieseman specializes in Dutch art, but her small informative guide, *Deceptions and Discoveries*, reveals that she is equally enthusiastic and knowledgeable about Italian and French paintings.

Several reassessments are impressive. Raffaello Raphael's *Portrait of Pope Julius II* has been upgraded from an early copy to the unique original, whereas a fine 15th-century group portrait turns out, in fact, to have been painted several hundred years later. Less surprisingly, scientists' grainy photographs and shadowy x-rays confirm technically what art historians already knew—even pictures carrying a famous signature may be collab-

orative workshop productions. On the other hand, Sandro Botticelli's *Venus and Mars* seems still more magnificent when placed next to a mediocre imitation for which (hard now to believe) the Gallery paid a higher price when acquiring them in the 19th century.

A free handout helpfully explains a range of technical artistic and scientific terms, such as craquelure, Raman microscopy, pentimento, and HPLC (high-performance liquid chromatography, used to analyze minute paint samples), and further details are provided in the catalog's 16 case studies. Lying beneath this surface varnish of technological whizz-kidder lies a murkier scene, a swirling chiaroscuro of marketing, valuation, and attribution. Revising assumptions of when a picture was painted, or of who wielded the paintbrush, can have dramatic effects on prices. A few years ago, the National Gallery launched a national appeal to prevent a recently discovered painting, Raphael's *Madonna of the Pinks*, from receiving an export license. This small canvas became a pilgrimage site,

even scientists sometimes opt for gut feelings. In his famous set of experiments, the American physicist Robert Millikan measured the charge of an electron by suspending electrified drops of oil, yet he discarded around two-thirds of his readings because he felt they were wrong. Despite this flagrant breach of protocol, Millikan was so attuned to the vagaries of his apparatus that he produced an answer remarkably close to the value accepted today.

When analyzing a painting, three main types of evidence come into play: documentary information about its origins, the appreciation of connoisseurs, and scientific data. As Wieseman explains, all three were important for assessing a small seascape by Jean Corot. The style and the scene both suggested that he painted it on a trip to Italy in 1826, but this dating was challenged when laboratory examination proved that he had used a viridian green paint available only several years later. To resolve this impasse, researchers eventually tracked down an earlier supplier in Paris, making it possible (although not proven) for the documentary, stylistic, and scientific facts to conform.

Unsurprisingly, reactionary art historians resent the intrusion by scientists who threaten their autonomy of connoisseurship and can upset the economy of the international auction trade by proving that an acclaimed

Close Examination Fakes, Mistakes and Discoveries

Marjorie E. Wieseman and Ashok Roy, Curators
National Gallery, London. Through 12 September, 2010.
www.nationalgallery.org.uk/

A Closer Look Deceptions and Discoveries

by Marjorie E. Wieseman
National Gallery, London, 2010. 96 pp.
Paper, \$15, £6.99. ISBN 9781857094862.



Not a copy. Infrared reflectogram of Caspar David Friedrich's *Winter Landscape* (probably about 1811) reveals an extensive underdrawing.



attracting admirers who had never given it a passing glance when hanging unrecognized in a country castle. This Italian painting of the Virgin Mary has little to do with British culture, yet a massive £22 million was raised to keep it in England, as if other nations were incapable of appreciating it.

The exhibition suggests that white-coated scientists inevitably provide all the right answers—but what counts as evidence? Quantitative arguments based on laboratory research usually trump intuitive hunches derived from experience, although

masterpiece is, in fact, a later copy. However, neither scientists nor curators seem to wonder whether validating a picture's genuineness is in itself a worthwhile exercise. Visitors to this exhibition are invited to spot the minute differences between pairs of virtually identical paintings, as though playing a game. But to give Roy's question a more contentious spin: if the only notable distinction is the number of zeros on the price tag, then does it really matter who painted a picture and when it was made?

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Partnerships for STEM Education

K. M. Foster,^{1,2*} K. B. Bergin,¹ A. F. McKenna,¹ D. L. Millard,¹ L. C. Perez,¹ J. T. Prival,¹ D. Y. Rainey,¹ H. M. Sevia,¹ E. A. VanderPutten,¹ J. E. Hamos¹

Schoolteachers and higher-education faculty can benefit one another to improve teaching and student learning.

As leaders in higher education, industry, and government (1) bemoan the limited academic success of students in science, technology, engineering, and mathematics (STEM), many practices of academe impede the ability of college and university faculty to address the issues. Consistent with barriers to community-engaged scholarship in general (2), STEM faculty engagement in elementary and secondary schools (K–12) can be undermined, for example, by (i) low status accorded to STEM education research and publications, (ii) a zero-sum view of faculty time allocation (e.g., K–12 engagement means time away from work more highly rewarded during promotion, tenure, and merit review), and (iii) bureaucracies that hinder collaboration between STEM faculty and K–12 teachers and administrators (3).

The U.S. National Science Foundation's Math and Science Partnership (NSF-MSP) program is taking steps to address such obstacles. Since 2002, the NSF-MSP has funded nearly 100 partnerships between institutions of higher education and K–12 school systems to build upon and generate knowledge of the impact of partnerships as a basis for STEM education reform, and about how to improve K–12 STEM outcomes (see the figure).

History

Individuals [e.g., John Goodlad (4)] and many university-based institutes and centers have implemented partnerships between higher education and K–12 schools while studying the process and outcomes. The NSF research-grounded support for higher education engagement in the K–12 arena was significantly enhanced with the post-Sputnik, 1957 passage of the National Defense Education Act, aimed at improving mathematics and science teaching. At their peak between 1960 and 1965, almost 1000 NSF teacher institutes were held each year for high school and elementary school teachers, with roughly 20,000 teachers attending (5, 6). Insights developed over the history of such



Collaborative instruction. (Top) Middle and high school teachers in the UTeachEngineering program at the University of Texas spend their summers learning engineering concepts they can use in their classrooms. (Bottom) Tiffany Knight, associate professor of Biology at Washington University in St. Louis (WUSTL), identifies invasive plant species with teachers in the Life Sciences for a Global Community MSP graduate degree program. Biology teachers (from left) are from Miami–Dade County Public Schools (Fla.), St. Louis Public Schools, and two from Barrington High School (Ill.). Two teachers in the background are unidentified.

efforts include (i) the need for research and evaluation through all stages of a project's life, (ii) the value of content and pedagogy for teacher effectiveness, (iii) the value of focusing not just on individual teachers but also on teachers' peer impact, and (iv) the importance of creating mechanisms for ongoing collaboration among teachers and with others (including higher-education faculty).

Well-planned, teacher learning experiences that involve STEM disciplinary faculty can deepen teacher content knowledge (7) and contribute to improved student outcomes (8). However, simply introducing STEM higher education faculty into K–12 settings does not guarantee positive results. There can

be downsides if this intervention is not properly planned and executed; if care is not taken in selecting participating faculty; and if potential limitations of faculty expertise, outside their disciplinary research, are not appreciated. This is an issue of concern over ineffective STEM teaching for higher education, as well (9).

Coming to Terms with the Barriers

NSF-MSP teams collect data, develop understanding, and, in some cases, implement strategies to facilitate and reward effective STEM faculty engagement in K–12 schools (10). As part of the statewide university system, Georgia's Partnership for Reform in Science and Mathematics (PRISM) engaged a cultural anthropologist to help determine what incentives and system-wide changes would motivate and support STEM faculty involvement in K–12 schools. Although the outcomes were not surprising—e.g., sustained faculty engagement would need systematic institutional support—they reflected specific local facets of the collaboration and provided concrete recommendations (11). Also at the state-system level, Maryland's Vertically Integrated Partnerships K–16 conducted a social network analysis to track connections among potential science education partners.

Activities such as vertically integrated learning communities, a teaching fellows program, and professional development workshops achieved immediate program goals but also facilitated relationships across the K–16 spectrum (K–12, plus college or university), including STEM faculty in particular. Connections among people interested in the same outcomes facilitate problem-solving, knowledge generation in areas of shared interest, and increased knowledge and effectiveness of individuals in the network (12).

Studies of campus cultures and policies help investigators discern rewards that are likely to facilitate STEM faculty participation. A study of eight NSF-MSPs found that

¹Division of Undergraduate Education, National Science Foundation, Arlington, VA 22230, USA. ²Institute for Community, University, and School Partnerships, The University of Texas at Austin, Austin, TX 78712, USA.

*Author for correspondence. E-mail: kmfoster@mail.utexas.edu

engaging faculty required that both their extrinsic and intrinsic needs be met (3). Extrinsic needs were met by some combination of stipends (typically summer salary) and releases from teaching responsibilities and intrinsic needs through intellectual connections that faculty members made with schools. Convening faculty and administrators to identify issues, needs, and rewards to promote faculty engagement, as well as to develop recommendations and strategies to see those recommendations through, can typically be handled within the existing structures of a given institution (13, 14).

Reforms

MSP projects have helped bring about tenure policy changes, prompted changes in institutional culture, and built incentives for faculty engagement. The El Paso Math and Science Partnership influenced the University of Texas at El Paso to adopt a new tenure and promotion policy, rewarding faculty engagement in K–12 education (15). A PRISM core strategy, built on long-standing K–16 reform work (13), contributed to a University System of Georgia Board of Regents statement advocating for work in schools (16). Further recommendations included revising the *Academic Affairs Handbook* and *Board of Regents Policy Manual* to ensure recognition for improving teaching and learning at the postsecondary level.

Structural reform efforts by faculty engaged in K–12 education research are consistent with broader efforts led by several professional organizations. For example, the Association of Public and Land Grant Universities (APLU) is undertaking an NSF-supported initiative that includes 123 universities committed to increasing the amount and diversity of mathematics and science teachers and to building partnerships to assess and meet state needs. At the institutional level, APLU convenes university leaders to address practices that might enable STEM faculties' engagement in teacher preparation, support, and development. At the faculty level, APLU is working with the American Physical Society and the American Chemical Society, associations with commitments to involving members in increasing the number of teachers in their disciplines.

Mutual Benefits

Higher education faculty and K–12 teachers bring different attributes and work as colearners and colleagues. Higher education faculty contribute additional content knowledge to complement the instructional expertise of K–12 teachers (17). STEM faculty

and K–12 teachers have coconstructed professional development experiences, resulting in increased self-efficacy in science understanding among teachers, shifts toward inquiry-based teaching, and improved test scores for students (18). Higher education faculty have worked with K–12 teachers to develop teacher content knowledge, pedagogy, and pedagogical content knowledge (i.e., tactics and strategies for effective teaching of specific concepts and skills).

Partnerships have also enlarged and strengthened the teacher corps. As part of the Boston Science Partnership (19, 20), between 2005 and 2010, 478 teachers from Boston area school districts have taken an average of 2.38 graduate science courses specially designed and offered with the development needs of the teachers and the schools in mind. Many have used these courses toward additional science teaching licensing. In 2005, Boston served a total of 57,000 students, but only 14 teachers in the district were licensed to teach high school physics. The partnership contributed to 272 teachers' adding new science licenses to their credentials as of 2009, with 170 of them in Boston and 28 of those in physics.

Whereas NSF-MSPs have primarily been funded to generate evidence on practices that will lead to improvements in K–12 STEM learning, benefits accrue to STEM faculty and higher education institutions, including improved university-level teaching, knowledge of K–12 education, and disciplinary research (21). Higher education faculty in the North Cascades and Olympic Science Partnership (NCOSP) used inquiry-based lessons in their own teaching more often than before their involvement in the partnership, elicited information from students to understand their preconceptions, and collaborated across institutions in their partnership to advance their work (8). Faculty found greater gains in students' learning in courses that they had reformed as part of their participation in the partnership versus students' learning in their traditional courses. Similar outcomes have been achieved among other projects in the MSP portfolio. Given the number of university courses affected (e.g., from 2003 to 2006, 329 undergraduate and graduate courses were developed or modified among 87 higher-education institutions participating in NSF-MSP), such findings could have implications for higher education if systematically applied.

As concerns mount over the college preparation and ongoing professional development of teachers, a tremendous opportunity exists to systematically address national STEM education imperatives. K–12 and

higher-education partnerships could allow systematic study and application of the collaborative work, for example, as STEM faculty gain teaching knowledge and apply it to higher-education courses. In the spirit of historical efforts to improve education in accordance with regional and national imperatives, the body of experience and literature on NSF-MSP and similar efforts could inform partnerships between institutions of higher learning and K–12 school systems as the centerpiece of STEM education reform.

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ASTRONOMY

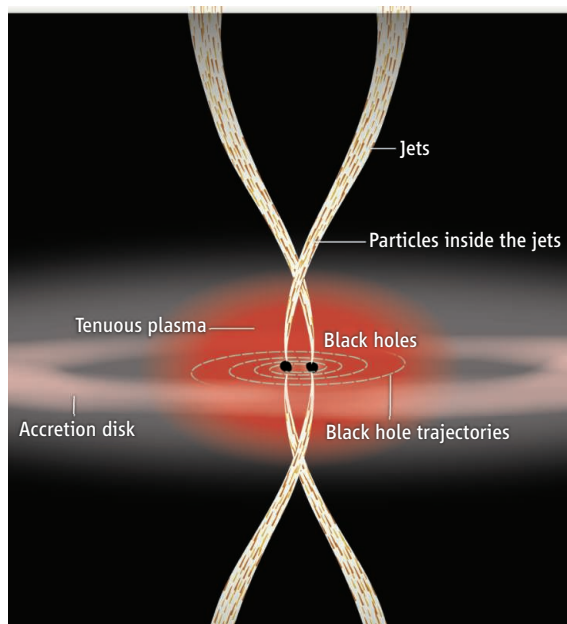
A Tale of Two Jets

Nicolás Yunes

One of the most astounding astrophysical phenomena is the existence of highly collimated jets of highly energetic particles found emanating from localized regions in the sky. These jets are ubiquitous, having been observed in the radio and x-ray spectrum at the center of galaxies, gamma-ray bursts, and quasars. Although their precise generation mechanism remains unknown, these jets are believed to be powered by black holes. Black holes do not shine in isolation, because their gravitational field is so strong that not even light can escape their pull. However, they are usually accompanied by rotating disks of gas and dust matter (called accretion disks) and can form strongly collimated emissions through a variety of mechanisms. On page 927 of this issue, Palenzuela *et al.* (1)

numerically model the merger of two black holes surrounded by an accretion disk that orbits around both of them (forming a circumbinary) and anchors a magnetic field perpendicular to the orbital plane. Each black hole generates a jet, produced by the twisting of the magnetic field lines as they lose energy and spiral toward each other (inspiral), even though the black holes are not spinning. Their simulations show the formation of two jets and their transition to a single jet as the black holes merge.

The seminal work of Blandford and Znajek (2) has helped us understand how jets form. They started by considering a single spinning black hole with a magnetized accretion disk, supported by external currents flowing in the disk. This disk generates a magnetic field that is dragged by the spinning black hole, inducing an electric field that accelerates stray electrons. The electron acceleration destabilizes the vacuum, forcing electron-positron pair production and the formation of a neutral plasma (with zero



A powerful merger. Illustration of two merging black holes in a circumbinary accretion disk. Each black hole possesses its own jet as a result of the stirring of magnetic field lines, anchored by the circumbinary disk. The jets get entangled during the late inspiral, and eventually merge as the black holes do.

net charge on average). This plasma is then believed to be funneled by the magnetic field lines rotating with the black hole, leading to the emission of synchrotron radiation. In this way, the accelerated plasma would generate a highly collimated electromagnetic flux that extracts rotational energy from the black hole. Nonlinear numerical simulations (3–7) have reproduced this scenario.

Supermassive black holes, however, do not exist in isolation; they have been observed at the center of most galaxies, which in turn have also been observed to undergo mergers with other galaxies. As these galaxies merge, the central black holes in each galaxy will sink to the center of the remnant, as a result of dynamical friction, and will form a binary system. While doing so, the accretion disks of each black hole combine into a circumbinary disk, which anchors a magnetic field in the inner region. This region is essentially devoid of material because the black holes evacuate it as they inspiral, as a result of loss of binding energy by gravitational wave emission. The black hole binary eventually merges, forming a single spinning black hole that is expected to possess a single Blandford-Znajek jet. Because of

Numerical models suggest that black hole mergers may produce a strong, potentially detectable electromagnetic counterpart to their emitted gravitational waves.

the strong nonlinearities and complex mathematics that describe this scenario, the standard analytical and perturbative techniques used in Blandford-Znajek-type calculations fail, obscuring the process through which such jets form.

Palenzuela *et al.* take advantage of recent advances in the numerical modeling of the merger of two nonspinning black holes in full general relativity (8–10). They include a magnetic field perpendicular to the orbital plane whose evolution can be described by Maxwell's equations coupled to the Einstein equations of general relativity. The magnetic field is supported by currents in the circumbinary disk, which decouples from the simulations as it is far from the binary. The electromagnetic field is treated in the so-called force-free approximation (2, 11), in which the bulk of the plasma is assumed to experience no force because of its negligible fluid inertia relative to the black holes' inertia.

These simulations reveal that even in the absence of black hole spins, dual jets are induced around each black hole by their orbital motion, and these jets merge into a single jet after the black holes merge (see the figure). Initially, when they are well separated, the black holes inspiral and stir the surrounding plasma and magnetic field lines. By electromagnetic induction, this motion produces an electric field oriented toward the poles, a toroidal magnetic field perpendicular to the electric field, and thus, the flow of electromagnetic energy, leading to dual jets (see the figure). Unlike in the Blandford-Znajek mechanism, the black holes are nonspinning and the jets are powered by the black hole's kinetic energy instead of their rotational energy. Eventually, they merge and form a highly distorted and spinning black hole, at which point a strong isotropic burst of electromagnetic radiation is emitted, with twice the amount of energy relative to the individual jets. In addition, the dual jet geometry transitions to a single, collimated jet that is well described by the Blandford-Znajek mechanism. These simulations thus prove that Blandford-Znajek jets are incredibly robust and stable, even in highly dynamical and asymmetric spacetimes, as in the merger of two black holes.

Apart from discovering a generalization of the Blandford-Znajek jet formation mechanism and elucidating the fate of

Department of Physics, Princeton University, Princeton, NJ 08544, USA, and Department of Physics and MIT Kavli Institute, MIT, Cambridge, MA 02139, USA. E-mail: nyunes@princeton.edu

dual jets in binary black hole mergers, the simulations of Palenzuela *et al.* also have strong implications for gravitational wave astrophysics. The electromagnetic waves produced according to this scenario should be detectable, as they present a very clear energy profile. During inspiral, the dual jets lead to a particular electromagnetic structure, lasting several days and with energies proportional to the magnetic field times the orbital velocity squared. Such emission remains roughly constant over most of the inspiral, as the orbital velocity varies slowly. When the binary merges, a strong isotropic burst of radiation is emitted and the dual jets transition to a Blandford-Znajek jet with its

own associated electromagnetic structure. If such a counterpart is observed, it would provide information on the location of the event in the sky. Such an electromagnetic observation could then be used in gravitational wave observations to break degeneracies and measure other astrophysical parameters more accurately, such as the Hubble constant (12) or the dark energy equation of state (13), or to test deviations from general relativity (14).

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CELL BIOLOGY

Nuclei Get TAN Lines

Daniel A. Starr

The intracellular localization of the nucleus within a cell is tightly controlled and plays a central role in numerous cellular and developmental processes, including the establishment of cell polarity, fertilization, differentiation, and cell migration. Defects in nuclear movement or anchorage can disrupt development and cause disease. Many nuclear migration events involve the microtubule cytoskeleton. The nucleus is often led by the centrosome (the structure that organizes microtubules), and microtubule motors are recruited to the nuclear envelope (1). Other migrations use the actin cytoskeleton (2), but the mechanisms of actin-based nuclear migration have not been clear. On page 956 of this issue, Luxton *et al.* describe an actin network that moves nuclei (3).

For a nucleus to move, its structural components must connect to force-generating structures in the cytoskeleton. This is complicated by the presence of a nuclear envelope that consists of two membranes. To link the nucleoskeleton (nuclear lamina) to the cytoskeleton, outer nuclear membrane KASH proteins and inner nuclear membrane SUN proteins form bridges across the nuclear envelope (1). Luxton *et al.* describe structures called transmembrane actin-associated nuclear (TAN) lines, which form such a bridge (see the figure). TAN lines are made of cytoplasmic actin cables, the KASH pro-

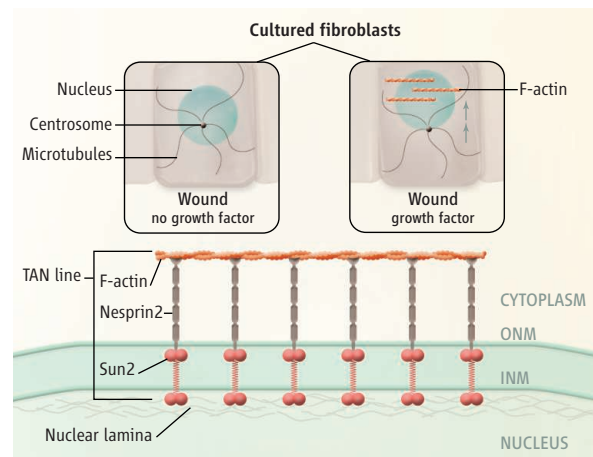
tein nesprin2, and the SUN protein Sun2. Mammalian nesprin2 (also known as Syne-2) is a gigantic protein (greater than 750 kD) that tethers the outer nuclear membrane to the actin cytoskeleton. It consists of an N-terminal actin-binding domain, multiple spectrin repeats that likely form a fiber, and a C-terminal KASH domain (4). The KASH domain spans the outer nuclear membrane and interacts with a SUN protein in the perinuclear space (5). A SUN protein spans the inner nuclear membrane and interacts with the nuclear lamina (6). The importance of KASH and SUN proteins has been demonstrated by genetic experiments in model

Positioning of the nucleus within a cell involves a protein complex that spans the nuclear envelope to connect actin filaments to the nuclear lamina.

organisms (1). ANC-1, the ortholog of nesprin2 in *Caenorhabditis elegans*, anchors nuclei to the actin cytoskeleton, and nesprin1 and nesprin2 function redundantly to position nuclei in mouse muscles and developing neurons (7, 8). Likewise, mutations in SUN proteins lead to similar developmental defects (8, 9).

The major limitation of the genetic studies implicating SUN and KASH proteins in nuclear positioning is the difficulty in observing nuclear movements in situ. Thus, Luxton *et al.* used a tissue culture system where nuclear migrations can be filmed (10). Mouse fibroblasts grown to confluency, wounded,

and exposed to a growth factor will polarize toward the wound edge. While microtubules and the motor protein dynein keep the centrosome in the center of the cell, actin retrograde flow moves the nucleus to a polarized location behind the centrosome. The advantages of this experimental system are that nuclear migration is independent of cellular migration, inducible, quantifiable, easily visualized, and amenable to disruption by small interfering RNA (siRNA) or the expression of mutant proteins. Luxton *et al.* used this system to show that nesprin2 and Sun2 are required for rearward nuclear migration in polarizing fibroblasts. Remarkably,



Moving with the SUN. TAN lines couple the nucleus to retrograde actin flow. Starved, confluent, and wounded fibroblasts are polarized after growth factor addition, which induces a rearward movement of nuclei. Microtubule networks (tan) remain centered while actin fibers (red) form on the dorsal surface of migrating nuclei. ONM and INM denote outer and inner nuclear membrane, respectively.

nuclear migration defects caused by reducing nesprin2 expression (by siRNA) could be rescued by a “mini” nesprin2 gene that includes the actin-binding and nuclear envelope-targeting domains but lacks the bulk of the spectrin repeats. This suggests that at least for polarizing fibroblasts, the length of nesprin2 is not important.

The strength of the Luxton *et al.* study is the use of live fluorescence microscopy to examine the localization and behavior of actin, nesprin2, and Sun2 during nuclear migration in cultured fibroblasts. It was previously observed that nuclear migration required the rearward flow of actin speckles (10). Luxton *et al.* now describe an array of actin cables that forms on the dorsal surface of nuclei, parallel to the wound edge, within 30 min after induction of polarization. The cables moved away from the wound edge at the same rate as nuclei. Both nesprin2 and

Sun2 were recruited to, and moved with, the actin cables to create TAN lines. TAN lines therefore contain all the components needed to bridge the nuclear envelope and couple the nuclear lamina to the cytoskeleton.

TAN lines have parallels to other complexes that connect actin assemblies to structural components on the opposite side of a membrane. For example, both focal adhesion complexes and the dystrophin complex span the plasma membrane, connecting actin filaments to the extracellular matrix. There are further similarities between nesprin2 and dystrophin—they have homologous actin-binding domains and spectrin repeats (2). In budding yeast, actin movements just outside the nucleus are coupled to rapid movements of chromosome ends (telomeres) during meiosis. A SUN protein is required for this coupling, which suggests that the assembly of TAN line-like structures at the nuclear sur-

face might be conserved (11). Future studies are required to determine whether TAN lines exist in three-dimensional tissues and how broadly such structures are used in the positioning of nuclei.

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PHYSICS

Directing Light Emission from Quantum Dots

Harald Giessen¹ and Markus Lippitz²

Cell phones, radios, and television sets contain antennas that pick up signals carried by electromagnetic radiation and convert them into pulses of electric current. Antennas connect two very different length scales—transmission wavelengths range from centimeters to meters, whereas component wiring and circuitry is on the micrometer-to-millimeter scale. On page 930 of this issue, Curto *et al.* (1) take this scaling concept to the optical world, where the interaction of light with matter includes quantum mechanics as well as classical electromagnetism. They fabricate nanoantennas from gold, a metal that can develop charge oscillations in its surface layers when excited by optical radiation. These antennas allow visible radiation, which has wavelengths of hundreds of nanometers, to couple into a semiconductor quantum dot only a few nanometers in diameter, and also direct the emission.

The value of a good antenna is best appreciated when a damaged antenna cable turns a TV picture into a snow pattern. A

temporary repair can be made by removing the faulty cable and sticking a screwdriver into the antenna plug. The reception will be somewhat grainy, but at least you can watch some programs. The reason for the poor picture quality is what electrical engineers call mode or impedance mismatch. The temporary antenna is not mode matched to the electronic circuits, so much of the signal fails to get into the circuits—it is reflected back into the screwdriver antenna. TV antennas are mode matched not only to the electronic circuitry, but also to electromagnetic waves, which travel through free space.

Antennas can be omnidirectional—for example, the simple dipole antenna that folds out from a portable radio. A very common TV antenna, the so-called Yagi-Uda antenna, invented by Yagi and Uda in 1926, is directional (see the figure, panel A). It can be tuned to pick up weak signals efficiently from distant transmitters. When used in transmitting mode, it can direct the outgoing beam into one direction about 5 to 10 times more efficiently than a dipole antenna.

Quantum emitters, such as atoms, molecules, and quantum dots, can also be regarded as extreme subwavelength “circuits.” An electrical engineer would regard them as rather

The photon emission from a semiconductor quantum dot directed by a nanoscale gold antenna could be used in quantum optics.

bad transmitters or receivers of radiation, because their extremely small size does not offer good mode matching to the dipole mode of the light to which they couple. Good mode-matched antennas reradiate their energy after excitation within a single cycle of the wave. Molecules or quantum dots take nanoseconds or even longer to reradiate their energy. This time scale corresponds to about 1 million oscillations at optical frequencies, and the emission is in all directions.

It is possible to fashion nanoantennas from metals such as gold that can bridge the mode-matching gap in the optical regime because they can reradiate their energy within femtoseconds (2). The very large oscillator strength of the antennas enables better coupling and faster response to the propagating dipole fields of free space compared to a quantum dot. Gold has a very high density of charge carriers—electrons and holes—oscillating back and forth at the so-called particle plasmon resonance. Each electron-hole pair can be viewed as a single dipole, and there are several millions of them in each nanoantenna of about 100-nm diameter.

If an atom, molecule, or quantum dot is placed into the near-field of a metallic

¹4th Physics Institute and Research Center SCoPE, University of Stuttgart, D-70569 Stuttgart, Germany. ²Max-Planck-Institute for Solid State Physics, D-70569 Stuttgart, Germany. E-mail: giessen@physik.uni-stuttgart.de

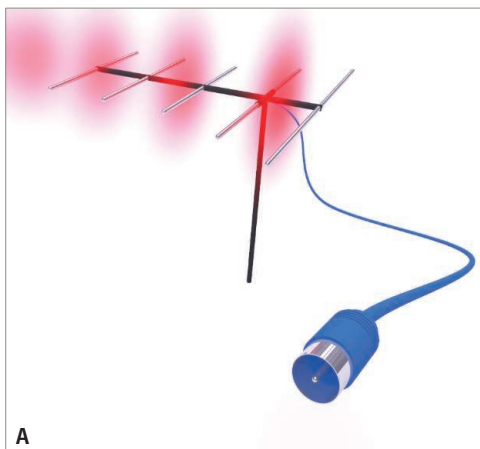
nanoantenna (within about 1/50th the wavelength of the emitted radiation), its excited state can radiate photons very efficiently to free space (see the figure, panel B). The quantum emitters can emit a single photon, which can be exploited in quantum optics. Additionally, the nanoantenna can redirect radiation into a defined solid angle in space and impose a specific polarization on it.

Until now, quantum emitters have mostly been coupled to rather simple structures, such as the metallic tip

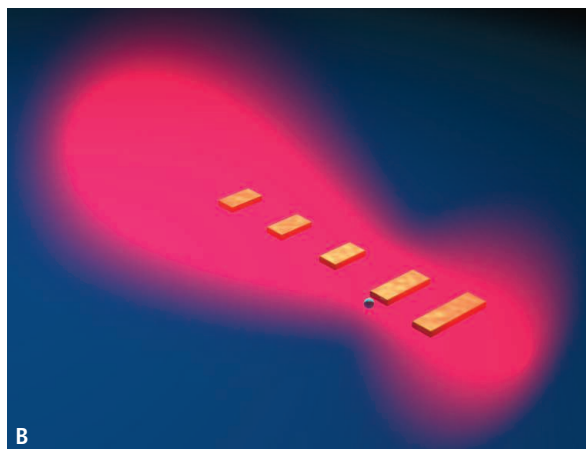
of a scanning probe microscope (3), simple metallic nanodots or nanowires (4, 5), gap antennas (6), or bowtie antennas (7). Curto *et al.* have manufactured metallic nanoantennas of different shapes, namely nanodots and nanowires, as well as directive Yagi-Uda antennas (8). They then positioned quantum dots near the antennas using an elaborate two-layer nanolithography scheme. The authors excited the quantum dots with a laser beam and observed blinking emission, a strong indication that only a single quantum dot was attached to the antenna. The emission pattern of the dot near the antenna is governed by the nanoantenna, as is the polarization of the emitted light. Especially convincing is their analysis of emitter coupling to the nanoantennas when the antenna's plasmon frequency matches that of the emitter (that is, it is in resonance, versus off resonance). By carefully preparing many different-sized structures, they show that the quantum dot emission is highly directional and polarized only under resonance conditions.

The approach developed by Curto *et al.* could lead directly to several important advances. Photon antibunching, in which photon emission is more equally spaced in time than with a light-emitting diode, could create an effective single-photon source for quantum communication that emits the single photons efficiently into a narrow beam. The demonstration of the Purcell effect, which is the acceleration of the decay of the quantum emitter caused by impedance matching by the antenna to free space, could also enhance the radiative emission over nonradiative losses.

The technology itself could be further improved, for example, by using an antenna array to gain even higher direc-



Antennas large and small. (A) A Yagi-Uda TV antenna is shown with feed, reflector, and directors, as well as its radiation pattern. The radiation is fed with very high efficiency into the antenna cable and connector, which are much smaller than the wavelength of the broadcast signal. (B) Curto *et al.* coupled the emission of a single quantum dot to a gold Yagi-Uda nanoantenna. This coupling increases the rate of photon emission and makes the emission pattern highly directional.



tivity or to scan the beam in several directions as is done with phased-array radar. Two nanoantennas could face each other to create an optical nanoantenna link (9) and, eventually, demonstrate antenna- or plasmon-based controlled coupling of two quantum emitters to show quantum interference. If the quantum emitter used was a nitrogen-vacancy center in diamond (10), a true room-temperature quantum computer could become a reality when exploiting the different spin states.

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GENETICS

Replication Error Amplified

Salma Kaochar, Andrew L. Paek, Ted Weinert

Yeast offers insight into how lifting controls on DNA re-replication can cause cells to make extra copies of a gene.

Cells have a seemingly endless array of regulators to provide for fast and accurate DNA replication. Most cells succeed in doing it right, each and every time they divide, which is testimony to the powers of evolution. Failure to limit DNA replication to just once every cell cycle can lead to genome rearrangements, in particular to “amplification,” or an increase in the number of copies of some genes. Amplification can drive abnormal cell growth, and cancer cells often have amplified oncogenes (1). How gene amplification occurs, however, is

unclear. One mechanism involves the breaking and fusion of aberrant chromosomes, a process first discovered by Nobel Laureate Barbara McClintock in the 1950s (2), which leads to extra copies of genes that are joined together (3). Another proposed mechanism involves defects in the “re-replication” controls that shut down the DNA-copying process after a single duplication, but this idea had not been testable (4). On page 943 of this issue, Green *et al.* overcome that hurdle with an extravagant experimental system in budding yeast that demonstrates that re-replication is indeed a potent mechanism of gene amplification (5).

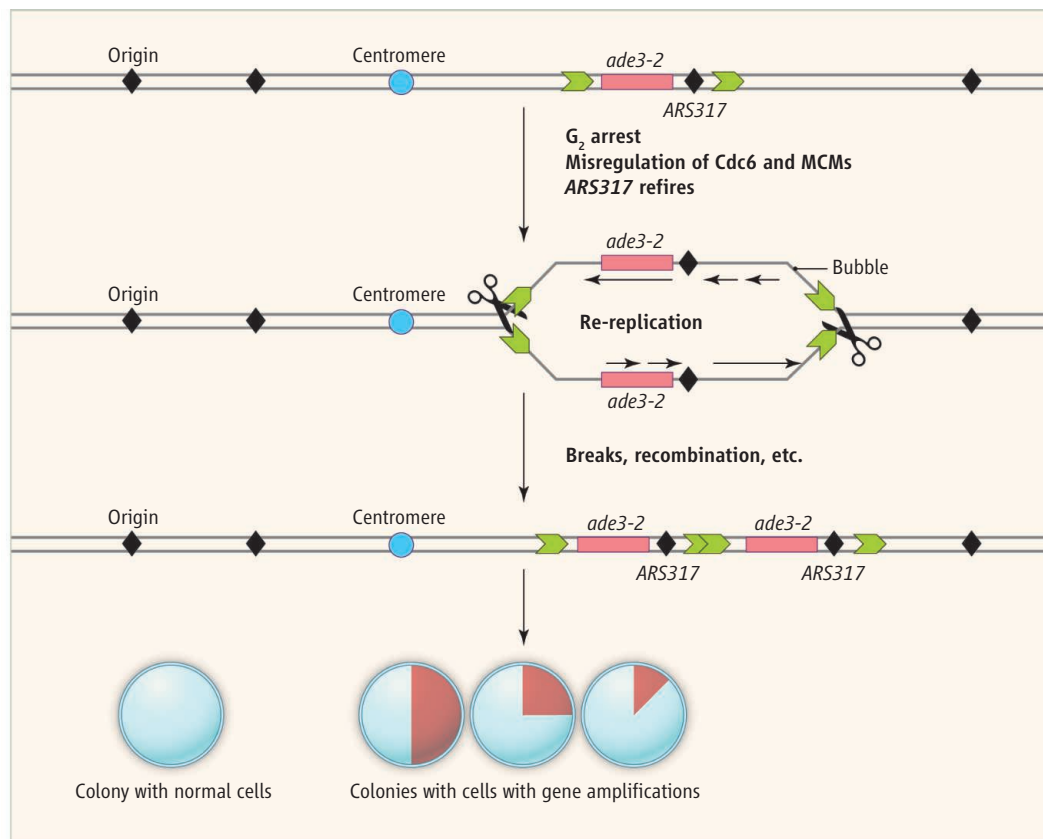
These authors have been studying the labyrinth of controls that normally prevent re-

Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, USA. E-mail: tweinert@email.arizona.edu

replication in budding yeast. In yeast, a key re-replication control involves cyclin-dependent kinase (CDK), a protein kinase that acts on multiple proteins at the “origins of replication,” some 300 discrete sequences of DNA at which copying is initiated (6). This is how it works: When CDK activity is low (in the M and G₁ cell cycle phases), proteins are assembled onto origins in preparation for replication. This preparation phase is called licensing. When CDK activity is high (in the S and G₂ phases), the licensed origins “fire,” or begin to replicate. A key to preventing re-replication is that high CDK activity blocks relicensing of fired origins; each origin thus can fire only once per cell cycle. Green *et al.* previously showed, however, that some origins can fire more than once in certain mutant yeast cells because of misregulation of several CDK substrate proteins associated with origins (Cdc6 and MCM helicase proteins) (7). They also observed that this refiring took place primarily at one origin, known as *ARS317*. This was a bit of good fortune, since having refiring occur only at a limited and well-defined region allowed easier interpretation of their results. Why *ARS317* refires so efficiently is unclear, but it is a terrific tool that Green *et al.* use to forge a strong link between gene amplification and re-replication.

Next, the authors used this system to ask whether re-replication of *ARS317* triggers gene amplification. They measured gene amplification using a convenient yeast trick: Cells with one copy of a selected gene (the *ade3-2p* allele) are pink, and cells with two copies are red (8). By simply looking at the color of yeast colonies, each containing a few million cells, Green *et al.* could determine whether gene duplication had taken place (see the figure). Using this system, they showed that deregulating re-replication increased gene amplification by a multiple of 230. Gene amplification was a very common outcome of re-replication; astoundingly, 1 out of every 20 cells that underwent re-replication underwent gene amplification.

Do the structure and genetics of the extra gene copies (amplicons) reveal any details of this mechanism? Structural analysis of the



A yeasty trick. In mutant yeast cells, misregulation of the Cdc6 and Mcm2-7 helicase proteins allows the *ARS317* origin (top) to fire more than once, enabling re-replication of DNA (center). In 1 of 20 cells, re-replication led to gene amplification of *ade3-2*, resulting from recombination of nearby repeat sequences (bottom). Increased copy number of *ade3-2* was indicated by the color of yeast colonies (circles), with red cells containing duplicate gene copies.

yeast amplicons revealed that they were usually tandemly arrayed in head-to-tail orientation, huge (>100 kb), and bound by repetitive DNA sequences (see the figure). Genetic analysis suggested that in the re-replicated cells, amplification is occurring independent of the breakage-fusion cycle that McClintock discovered 60 years ago (as evidenced by the observation that the activity of the ligase gene *Dnl4* is not required) and involves non-allelic homologous recombination (activity of *Rad52* is required). Certain details of how re-replication leads to amplification remain unclear. For example, do cells that have replication “bubbles”—structures that form at origins when proteins separate the DNA strands (see the figure)—complete additional rounds of cell division, with bubbles intact, before their resolution by recombination?

Given this direct mechanistic demonstration in yeast, it is tempting to speculate that re-replication also leads to gene amplification and other genomic alterations in cancer that occur in higher organisms. Making that connection in mammalian cells, however, relies on inference and correlation, as the genetic tricks possible in yeast are not yet possible

in mammalian cells. It is suggestive that, in certain tumors, Cdc6 and other regulators of re-replications are overexpressed (9); might their overexpression trigger re-replication and gene amplification? Or is their misregulation a consequence of tumor formation? There is as yet no solid evidence either way. Confoundingly, some new studies also suggest that misregulation of Cdc6 can cause genome alterations independent of detectable re-replication (10, 11). For Cdc6, Gonzalez and colleagues showed that Cdc6 facilitates heterochromatinization (inactivation of normally active euchromatin) and gene silencing of oncogenes in certain tumor cells, activities that are independent of re-replication (11). Because of the poor sensitivity of the available re-replication assays, it remains unclear whether re-replication also contributes to genomic instability in these tumor cells. Hence, the role of re-replication in gene amplification and tumor formation remains to be clarified.

Finally, the yeast study provides a solid example of another curious and perhaps relevant feature of DNA structure. Because of its propensity to undergo re-replication (at

least in mutants), the *ARS317* region can be considered a “hot spot” in the yeast genome for amplification. In human tumors, there also appear to be “hot spots” of gene amplification (12). It is not clear whether these recurrent duplications are due to inherent instability (re-replication?) of specific origins, or are simply due to a selective advantage that duplication provides. The extent to which re-replication-driven insta-

bility explains other unusual DNA structures common in cancer cells—such as the small fragments of extrachromosomal DNA known as “double minutes”—also remains to be clarified.

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NEUROSCIENCE

A Glutamate Pathway to Faster-Acting Antidepressants?

John F. Cryan and Olivia F. O'Leary

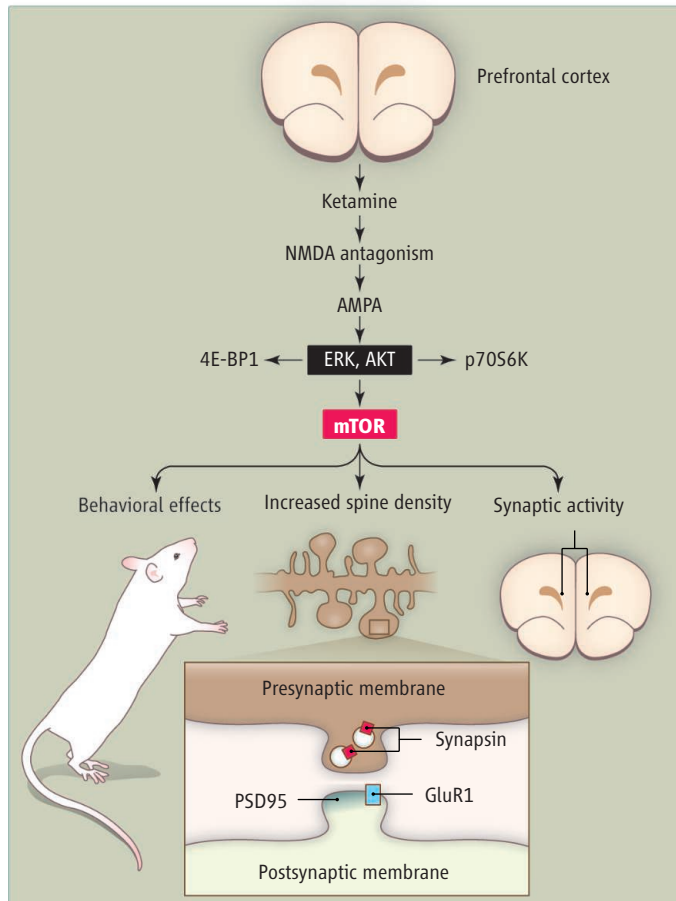
Depressive illness was described by Hippocrates in ancient Greece, but effective therapeutic agents did not emerge until the 1950s. Today, almost all antidepressant drugs in clinical use increase levels of certain neurotransmitters in the brain, in particular norepinephrine and serotonin. Although these medications are beneficial, a sizeable minority of patients remain resistant to their therapeutic effects (1). Moreover, in most patients, there is a delay of weeks to months before the drugs take full effect. As a result, there is an urgent need to develop faster-acting drugs (2–4).

One approach has rested on the hypothesis that current drugs facilitate a gradual reorganization of the connections (synapses) and communication between neurons, perhaps by integrating new brain cells into neural networks or by increasing the turnover of synapses and their related proteins (5–8). Glutamate, the brain's major excitatory neurotransmitter, is involved in these processes. In the last decade, several clinical studies have reported that a single subanaesthetic dose of ketamine, which blocks a particular cell receptor for glutamate (the NMDA receptor), resulted

in a rapid antidepressant effect within hours of administration, and that this effect could be sustained for at least 1 week (9, 10). Ketamine also improved depressive symptoms in treatment-resistant patients (10, 11). In addition, a single dose produced a rapid and sustained antidepressant effect in several animal models (12).

Activation of mTOR, a ubiquitous protein, in the prefrontal cortex could be a key goal of new drugs.

On page 959 of this issue, Li *et al.* (13) take a potentially important step toward identifying faster-acting antidepressants by identifying the cellular signaling pathways in the prefrontal cortex of the rat brain that are rapidly activated by ketamine. A single dose increased expression of synaptic proteins within 2 hours and increased dendritic spine density and synaptic activity within 24 hours (12). One of the pathways activated by ketamine was the mammalian target of rapamycin (mTOR), a ubiquitous protein kinase involved in protein synthesis and synaptic plasticity (14). By directly administering rapamycin, which inhibits mTOR, into the brain, they demonstrated that mTOR is required for ketamine-induced increases in synaptic proteins, dendritic spines, and some forms of synaptic activity in the prefrontal cortex. Moreover, inhibition of mTOR in the medial prefrontal cortex, a specific brain



Fast action. In rats, a single dose of ketamine was sufficient to activate the mTOR pathway in the prefrontal cortex, increasing the expression of synaptic proteins and the density of dendritic spines and inducing an antidepressant response within a day. AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; ERK, extracellular signal-regulated kinase; GluR1, AMPA receptor subunit 1; mTOR, mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate; p70S6K, p70S6 kinase.

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School of Pharmacy, Department of Pharmacology and Therapeutics, Alimentary Pharmabiotic Centre, University College Cork, College Road, Cork, Ireland. E-mail: j.cryan@ucc.ie

region thought to be affected by stress and depression (15, 16), prevented ketamine from producing its antidepressant-like behavioral effects in several animal models. Notably, in one behavioral test, the animals responded to a single dose of ketamine; typically, the animals require repeated doses of current antidepressants to show a response in this test.

Li *et al.* also demonstrated that mTOR is activated by another compound, Ro 25-6981, which selectively acts on a certain type of NMDA receptor. Ro 25-6981 also increased the expression of several signaling and synaptic proteins on time scales similar to those of ketamine. Moreover, a single administration of Ro 25-6981 produced antidepressant-like behavioral effects that were also dependent upon mTOR signaling. Interestingly, the mTOR pathway was not activated by single or repeated doses of two commonly used antidepressant drugs or by electroconvulsive shock, which is one of the most effective therapies in treatment-resistant depression.

Together, these findings suggest that the rapid activation of mTOR-mediated signaling pathways may be an important and novel strategy for the rational design of fast-acting antidepressants. The exciting results also demonstrate that ketamine may be a useful tool to identify molecular mediators of rapid antidepressant effects. Neither ketamine nor mTOR, however, is the Holy Grail

of rapid antidepressant therapy. Ketamine can cause symptoms that mimic psychosis and can cause cognitive impairment, limiting its potential for widespread clinical use. Because mTOR is a ubiquitous enzyme that regulates protein synthesis, treatments that fail to appropriately restrict mTOR signaling to specific times and parts of the brain could have detrimental effects in humans, such as cancer (17).

Although the present study demonstrates that mTOR activity, specifically in the medial prefrontal cortex, is critical to the antidepressant effects of ketamine, past studies have found that global inhibition of mTOR also has antidepressant-like behavioral effects (18). It remains unclear, however, whether ketamine and antidepressants alter mTOR activity in other brain regions also affected in major depression. The expression and activity of mTOR in the depressed human brain is unknown, and caution must always be taken when comparing results from rodents to humans, who have a much more elaborate frontal cortex (15). Clinical trials of other compounds that act on NMDA receptors are under way (19, 20). As their results emerge, we now have a potential cellular mechanism to explain how these glutamate ligands induce their rapid antidepressant action. This may brighten the outlook for antidepressant drug discovery.

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IMMUNOLOGY

Double TIP-ping

Harinder Singh^{1,2} and Ignacio A. Demarco¹

Bcells of the mammalian immune system use two different means of shuffling gene segments to generate antibodies that recognize pathogens. Unique variable regions of the heavy- and light-chain subunits that constitute an antibody are genetically encrypted through DNA recombination during B cell development (1). The process generates millions of naïve B cell clones, each expressing a distinct antibody whose variable portion recognizes a particular antigen of a pathogen. Upon recognizing a specific antigen, a naïve B cell differentiates into an antibody-secreting plasma cell by further shuffling gene segments that encode the constant region of the heavy chain, while holding the variable region fixed (2). This

process, called class switch recombination, is initiated by the enzyme activation-induced cytidine deaminase (AID) (3). On page 917 of this issue, Daniel *et al.* (4) identify a dual actor in the molecular steps that precede and follow AID action, thereby enabling class switch recombination and refinement of the antibody repertoire.

Each immunoglobulin heavy-chain (IgH) constant region is associated with a cis-acting regulatory DNA sequence called a switch (S) region (see the figure), where a double-strand break (DSB) in DNA is initiated by AID. Accessibility of the S region to AID depends on its transcription. The RNA polymerase II (Pol II) complex transiently generates single-stranded DNA, the preferred substrate for AID, by unwinding helical double-stranded DNA (2). During B cell differentiation, transcription factors bind to promoter sequences near S regions and are thought to load AID

A protein induces gene activation marks in chromatin and repairs DNA breaks to promote genetic recombination during antibody class switching.

onto RNA Pol II, allowing the former to “piggyback” into the S region (5). AID action at an S region involves cytidine deamination. Uracil base excision and cleavage of abasic sites by DNA repair enzymes culminates in nicks in single-stranded DNA that are converted into DSBs. DSBs activate the non-homologous end joining pathway (NHEJ), which ligates S-region ends, yielding an allele that encodes a heavy-chain subunit with a switched constant region (6). These considerations led Daniel *et al.* to focus on Pax transcription activation domain interacting protein (PTIP). PTIP is implicated in NHEJ and also associates with a histone methyltransferase that functions in gene activation (7, 8). The question arose as to whether PTIP might regulate class switch recombination by activating S-region transcription as well as by joining of DSBs, thereby acting before and after AID, respectively.

¹Department of Discovery Immunology, Genentech, San Francisco, CA 94080, USA. ²Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637, USA. E-mail: singh.harinder@gene.com

Daniel *et al.* deleted the gene encoding PTIP in mouse B cells in vivo and then activated them with bacterial lipopolysaccharide (LPS) in vitro to stimulate class switch recombination. LPS induces AID expression as well as the transcription of particular S regions. The S μ region is active in naïve B cells, whereas the transcription of the other S regions is tightly regulated by distinct signals. This enables B cells to control the immunoglobulin isotype (determined by the constant region of the heavy chain) that they produce. Daniel *et al.* observed that B cells lacking PTIP were defective in certain class switch recombination events and in activating transcription through the corresponding S regions. Using chromatin immunoprecipitation coupled with DNA sequencing, the authors demonstrated that the absence of PTIP results in loss of methylation of

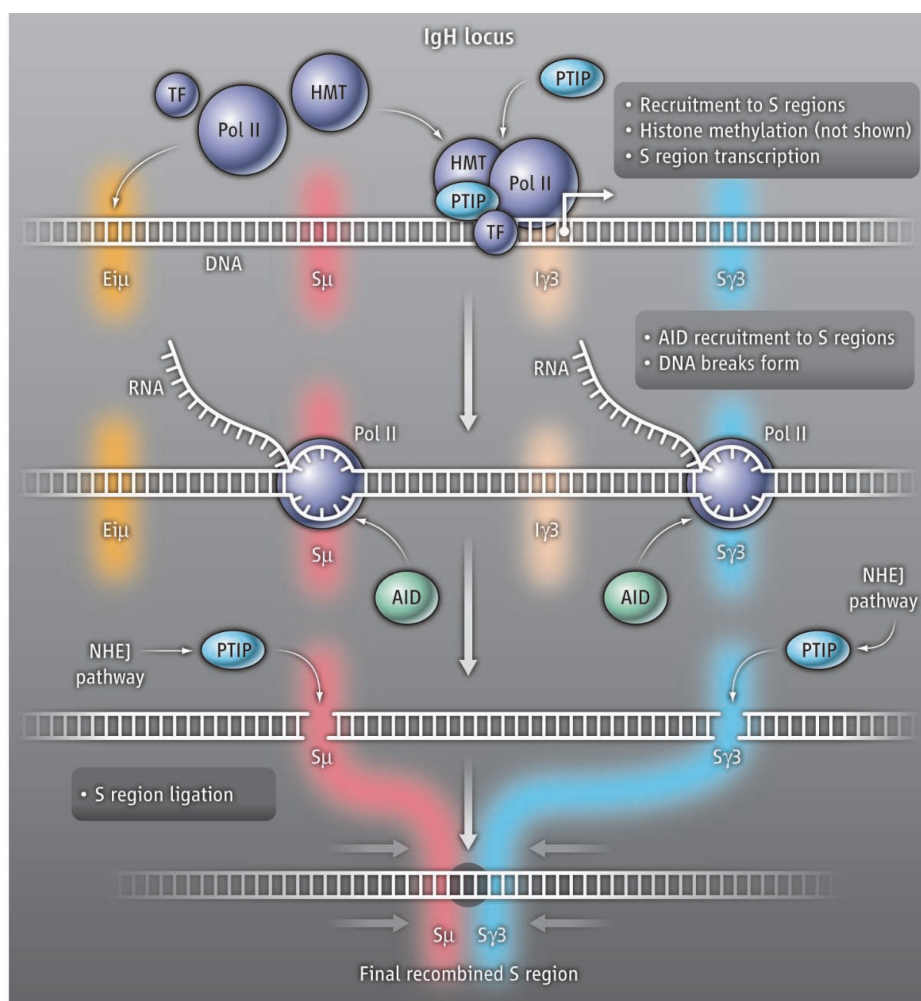
histone 3 at lysine 4 (H3K4me) at these S regions. This histone modification promotes gene activation.

Additional chromatin analyses revealed that subunits of a histone methyltransferase associate with DNA near the promoters of these S regions. The authors infer that complexes containing PTIP and histone methyltransferase subunits are targeted to specific S-region promoters through the physical interaction of PTIP with transcription factors that bind to these regulatory regions. The ensuing histone modifications facilitate the accessibility and recruitment of RNA Pol II to these S-region promoters. The identities of the transcription factors that target PTIP-containing histone methyltransferase complexes to particular S-region promoters remain to be determined. The transcription factor Pax5 is an attractive candidate given that PTIP was

originally identified as interacting with a related family member, Pax2 (7). Furthermore, Pax5 plays key roles in B cell development and activation, including the induction of AID expression (9).

To analyze the role of PTIP in class switch recombination following the action of AID, the authors complemented B cells lacking PTIP with the wild-type protein or a mutant version that does not interact with NHEJ pathway components or form foci at sites of DNA damage. Whereas the wild-type protein fully rescued the class switch recombination defects, the mutant protein did so partially despite restoring normal levels of S-region transcription. Together with the observation that PTIP suppresses genomic instability in B cells, this result suggests an additional role for PTIP in joining DSBs during a DNA damage response as well as class switch recombination. This is consistent with the requirement of another factor in NHEJ and also class switch recombination, which recruits PTIP to DSB repair foci (8, 10). The precise molecular actions of PTIP in the NHEJ pathway remain to be elucidated.

The duality of PTIP function in transcriptional activation through its interaction with transcription factors and histone methyltransferase subunits, and in the NHEJ pathway through its association with DSB repair components, appears to reside in distinct protein domains. Thus, PTIP can be viewed as a multifunctional adaptor that regulates gene activation as well as DNA recombination and repair. The mammalian adaptive immune system appears to have co-opted this ubiquitously expressed protein that functions in fundamental pathways of gene activation and the maintenance of genomic stability to control a specialized process of DNA recombination that diversifies the effector functions of antibodies.



Antibody class switching. The schematic depicts how PTIP regulates DNA recombination during a switch from an IgM isotype to IgG3. Transcriptional regulatory elements (E, I) and switch (S) regions are shown. Transcription factors (TFs) bind to E μ and upstream of I γ 3 and recruit histone methyltransferase (HMT) complexes. PTIP helps recruit HMT to I γ 3. HMTs promote histone methylation (not shown) and transcription of S regions by RNA Pol II. AID associates with RNA Pol II and catalyzes DNA double-strand breaks (DSBs). PTIP is proposed to facilitate synapsis and/or ligation of the DSBs in conjunction with the NHEJ repair pathway.

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Land-Level Changes Produced by the M_w 8.8 2010 Chilean Earthquake

Marcelo Farías,^{1*} Gabriel Vargas,¹ Andrés Tassara,² Sébastien Carretier,³ Stéphane Baize,⁴ Daniel Melnick,⁵ Klaus Bataille²

The 27 February 2010 [moment magnitude (M_w) 8.8] south-central Chilean earthquake was the fifth largest event recorded by modern seismology. We present field measurements of coseismic land-level changes from 24 sites along the coast and 9 sites at estuarine valleys (Fig. 1). A white fringe formed by a dead coralline crustose algae (*Lithothamnium*), raised above the lower intertidal zone, provides a direct measure of uplift (1); whereas submerged anthropogenic markers and the lower limit of vegetation indicate subsidence (fig. S1) (2). Estimated vertical displacements agree with preliminary Global Positioning System (GPS) results (3).

The largest uplift of up to 2.5 m occurred in the Arauco Peninsula (37.1°S to 37.7°S), where

marine platforms emerged, shifting the coastline up to 0.5 km toward the ocean (fig. S1). Detectable displacements occurred between 34°S and 38°30'S, in coincidence with the first hour of aftershocks (Fig. 1, A and B, and fig. S2). During the following hours, seismicity expanded to the north and south, covering an area between 33°S and 39°S. We propose that the maximum length of coseismic rupture is confined between 34°S and 38°30'S (~500 km), in agreement with preliminary slip models (4–6). Aftershocks outside this area would be associated with an accommodation of stress changes induced by the mainshock on adjacent segments of the megathrust fault.

The trench-perpendicular trend of vertical displacements reveals a hinge line located 118 ±

2 km inland from the trench (Fig. 1C). The uplift-subsidence pattern is well fitted by a simple elastic dislocation model that considers uniform slip on a rectangular fault (Fig. 1C and table S2). This is a first-order approximation to fault source parameters, and the scattering of our data with respect to the model suggests that the slip was spatially variable. However, parameters for the best-fitting model give $M_w = 8.81$ (2), which is equivalent to that reported by the National Earthquake Information Center (7).

Considering the current plate convergence [6.8 cm/year (8)], our modeled slip (10 m) is slightly lower than the 11.9 m expected from full plate coupling since the last earthquake in this region (175 years ago). This and the closely reverse pattern of coseismic displacements with respect to a geodetically constrained interseismic model (8) suggest that most of the strain accumulated during the seismic cycle was elastically released by the 27 February earthquake.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S3

Tables S1 and S2

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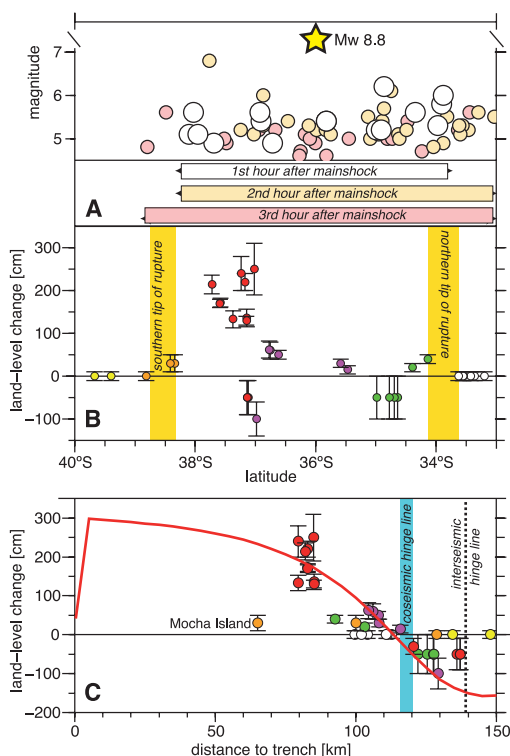


Fig. 1. Land-level changes along and across strike and their spatial correlation with aftershocks. (A) Aftershocks ($M_w > 4.5$) during the first 3 hours (8). (B) Vertical displacements along latitude. Colors identify different latitudinal segments. Error bars indicate the measurement uncertainties (2). (C) Vertical displacements versus distance perpendicular to the trench. The color scale is the same as in (B). The correlation between (A) and (B) suggests that the maximum length of rupture is confined between 34°S and 38°30'S. In (C), the smaller uplift at Mocha suggests a lower displacement at the southern tip of the rupture region or elastic accommodation south of the rupture area. Thus, uncertainties on the rupture length are emphasized in the width of the yellow box in (B). The red line in (C) corresponds to vertical displacements predicted by an elastic dislocation model based on Okada (9) [details in (2) and table S2]. This model considers a fault dip of 18° (10), the depth to the updip and downdip limit of rupture as 0 and 43 km, respectively, and a uniform coseismic slip of 10 m (2).

¹Departamento de Geología, Universidad de Chile, Plaza Ercilla 803, Santiago, Chile. ²Departamento de Ciencias de la Tierra, Universidad de Concepción, Casilla 160-C, Concepción, Chile. ³Institut de Recherche pour le Développement (IRD), Laboratoire des Mécanismes et Transferts en Géologie, Université Paul Sabatier (Observatoire Midi-Pyrénées), Université de Toulouse, 14, Avenue Belin, Toulouse 31400, France. ⁴Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Boîte Postal 17, 92262 Fontenay-aux-Roses, France. ⁵Institut für Erd- und Umweltwissenschaften, Universität Potsdam, Haus 27, Zi. 2.26, Karl-Liebknecht-Straße 24, 14476 Golm, Germany.

*To whom correspondence should be addressed. E-mail: mfarías@dgf.uchile.cl

PTIP Promotes Chromatin Changes Critical for Immunoglobulin Class Switch Recombination

Jeremy A. Daniel,¹ Margarida Almeida Santos,^{1*} Zhibin Wang,^{2*} Chongzhi Zang,^{3*} Kristopher R. Schwab,⁴ Mila Jankovic,⁵ Darius Filsuf,¹ Hua-Tang Chen,¹ Anna Gazumyan,⁵ Arito Yamane,⁶ Young-Wook Cho,⁷ Hong-Wei Sun,⁸ Kai Ge,⁷ Weiqun Peng,³ Michel C. Nussenzweig,⁵ Rafael Casellas,⁶ Gregory R. Dressler,⁴ Keji Zhao,² André Nussenzweig^{1†}

Programmed genetic rearrangements in lymphocytes require transcription at antigen receptor genes to promote accessibility for initiating double-strand break (DSB) formation critical for DNA recombination and repair. Here, we showed that activated B cells deficient in the PTIP component of the MLL3 (mixed-lineage leukemia 3)–MLL4 complex display impaired trimethylation of histone 3 at lysine 4 (H3K4me3) and transcription initiation of downstream switch regions at the immunoglobulin heavy-chain (*Igh*) locus, leading to defective immunoglobulin class switching. We also showed that PTIP accumulation at DSBs contributes to class switch recombination (CSR) and genome stability independently of *Igh* switch transcription. These results demonstrate that PTIP promotes specific chromatin changes that control the accessibility of the *Igh* locus to CSR and suggest a nonredundant role for the MLL3–MLL4 complex in altering antibody effector function.

Mixed-lineage leukemia (MLL)–like complexes catalyze mono-, di-, and trimethylation of histone H3 at lysine 4 (H3K4me), marks which all strongly correlate with active gene expression (1), but the direct role and specificity of these complexes during lymphocyte maturation are unknown. The PTIP (Pax interaction with transcription-activation domain protein-1, also known as Paxip1) protein stably associates with a subset of MLL-like complexes containing the MLL3 and MLL4 methyltransfer-

ases (2–4), suggesting a role for PTIP in transcriptional regulation. Embryonic lethality of *Paxip1*^{−/−} mice at embryonic day 9.5 (5), however, has precluded any further elucidation of its biological function, with the exception of a role in adipogenesis (6).

Along with possible functions in transcriptional regulation, PTIP contains six BRCT (BRCA1 carboxy terminal) domains, implicating the protein in the DNA damage response (7). Indeed, upon treatment with ionizing radiation (IR), PTIP

forms nuclear foci dependent on the γ -H2AX/MDC1/RNF8 damage response pathway and its own BRCT function, and also physically interacts with the DNA repair factor 53BP1 (7–9). PTIP-deficient cells are also hypersensitive to irradiation (9–11) and defective in homologous recombination (12). To what extent PTIP functions directly in double-strand break (DSB) repair, however, is unclear because loss of PTIP is also associated with defective proliferation (5) and a global decrease in H3K4me3 (3, 13), a chromatin mark of transcription initiation (14–16).

During V(D)J recombination in developing B cells in the bone marrow, recombination-activating gene 1/2 (RAG1/2)–mediated cleavage and DSB repair by the nonhomologous end-joining (NHEJ) pathway occur at accessible recombination signal sequence chromatin in antigen receptor genes and require actively transcribed gene elements (17). In response to ligand engagement from lipopolysaccharide (LPS), B

¹Experimental Immunology Branch, National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD 20892, USA.

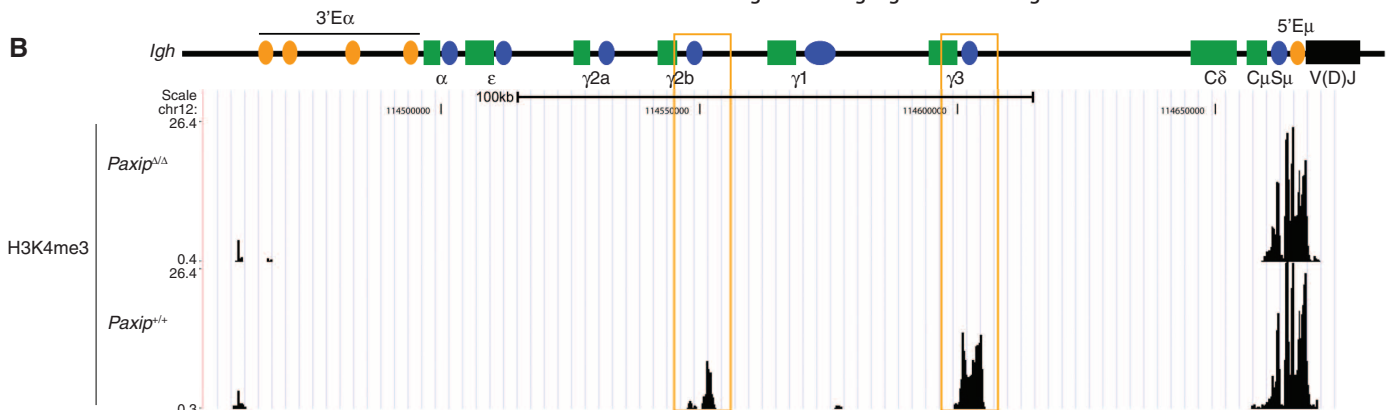
²Laboratory of Molecular Immunology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892, USA. ³Department of Physics, The George Washington University, Washington, DC 20052, USA. ⁴Department of Pathology, University of Michigan, Ann Arbor, MI 48109, USA. ⁵Laboratory of Molecular Immunology, The Rockefeller University, and Howard Hughes Medical Institute, New York, NY 10065, USA. ⁶Genomics and Immunology, National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIH, Bethesda, MD 20892, USA. ⁷Nuclear Receptor Biology Section, CEB, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA. ⁸Biodata Mining and Discovery Section, NIAMS, NIH, Bethesda, MD 20892, USA.

*These authors contributed equally to this work. [†]To whom correspondence should be addressed. E-mail: andre_nussenzweig@nih.gov

A Regions displaying PTIP-dependent H3K4me3

Region	Fold change
<i>Igh</i> - γ 2b switch region	30.5
<i>Igh</i> - γ 3 switch region	11.4
<i>Paxip</i>	8.9
<i>Slit3</i>	7.0
mRNA AK171983/ <i>Cacna1e</i>	4.9
<i>Il6</i>	4.0

Fig. 1. Genome-wide H3K4me3 changes in LPS-stimulated *Paxip*^{Δ/Δ} B cells. **(A)** Table showing average fold change decrease of most affected regions from three independent H3K4me3 ChIP-Seq experiments with multiple mice. Values represent control/*Paxip*^{Δ/Δ} ratios of sequence tag counts. **(B)** H3K4me3 ChIP-Seq profiles across the *Igh* constant region locus in B cells stimulated with LPS and α -IgD-dextran for 2 days. Data are representative of three independent experiments. In the illustration, green rectangles indicate constant (C) region exon segments, blue circles indicate switch (S) regions, orange ovals indicate enhancers (E), and the black rectangle indicates the antigen recognition V(D)J gene segment. The μ , δ , γ 3, γ 1, γ 2b, γ 2a, ϵ , and α isotypes correspond to immunoglobulins M, D, G3, G1, G2b, G2a, E, and A. LPS-induced switch regions are highlighted with orange boxes.



cell receptor cross-linking, and/or cytokine stimulation, mature naïve immunoglobulin M (IgM)-expressing B cells in the periphery proliferate and become activated to undergo class-switch recombination (CSR) (17, 18). This second rearrangement event promotes recombination of an antigen recognition gene segment with different *Igh* constant regions to interact with different cell surface receptors for successful clearance of a pathogen (17, 18). During CSR, transcription of immunoglobulin heavy-chain (*Igh*) switch region chromatin is required for accessibility of AID (activation-induced cytidine deaminase) (19, 20), which leads to formation of DSBs both at the upstream switch (S_μ) region and one of the downstream switch regions (S_γ3, S_γ1, S_γ2b, S_γ2a, S_ε, or S_α) (18). Subsequently, synapsis and DNA repair of the two switch regions are mediated by γ-H2AX/53BP1 and NHEJ proteins, leading to a recombined *Igh* locus (21). Given that DNA rearrangements in B lymphocytes require transcription and DNA repair at immunoglobulin genes and that PTIP is implicated in both of these processes, we investigated PTIP function in CSR.

Genome-wide H3K4me3 in LPS-stimulated *Paxip*^{Δ/Δ} and control B cells. To determine how changes in histone H3K4 methylation regulate mature B cell differentiation, we used chromatin immunoprecipitation coupled with Illumina sequencing (ChIP-Seq) to compare genome-wide H3K4me3 profiles of resting murine B

cells with B cells stimulated with LPS and antibody against IgD conjugated to dextran (αIgD-dextran, herein referred to as LPS stimulation), mitogens known to induce rapid proliferation and activation *ex vivo*. We observed that 1014 regions across the genome display more than a fourfold increase in H3K4me3 after LPS stimulation (fig. S1A and table S1). Only 13.2% of these LPS-induced H3K4me3 regions were localized within 4 kb of known transcriptional start sites, suggesting that LPS stimulation may initiate transcription at many uncharacterized genomic regions (fig. S1B).

Several genes whose transcripts are LPS inducible in B lymphocytes (18) displayed increased H3K4me3 upon LPS stimulation, including *Il6*, the *Igh* switch regions, and *Aicda* (encodes activation-induced cytidine deaminase) (fig. S1, C to E). In contrast, *c-myc*, which is induced during B cell activation but displays promoter-proximal polymerase II (Pol II) pausing (22), displayed similar H3K4me3 profiles in both resting and LPS-stimulated B cells (fig. S1E). These data support the notion that although *c-myc* may be poised for induced expression in mature B cells, *Aicda*, *Il6*, and the *Igh* switch regions are devoid of H3K4me3 until B cell activation, thereby comprising a different group of tightly regulated genes that have predominant control mechanisms that normally prevent initiation (15).

To investigate the genetic requirements and biological impact of LPS-induced H3K4me3, we crossed *Paxip*^{fl/fl} mice with CD19-cre mice to gen-

erate B cell-specific PTIP knockout mice. Analysis of *Cd19-cre Paxip*^{fl/fl} splenic B cells (herein referred to as *Paxip*^{Δ/Δ} B cells) revealed reduced PTIP protein and transcript expression while displaying near-normal CD19⁺ B cell numbers and frequency of surface IgM⁺ cells (fig. S2, A to D). In contrast to primary fibroblasts and embryonic stem cells (5), PTIP-deficient B cells proliferated in a manner similar to that of controls in response to either LPS or LPS and interleukin-4 (IL-4) (fig. S2E). Together these data suggest that PTIP is largely dispensable for B cell development and cell cycle progression.

Normal proliferation of *Paxip*^{Δ/Δ} B cells suggested the possibility of a more limited role for PTIP-mediated histone methylation in B cells compared to the global reduction of H3K4me3 observed in developing tissues (3, 13). We found that only six regions (*Igh-γ*2b, *Igh-γ*3, *Slit3*, *Il6*, *Paxip1*, and *mRNA AK191783/Cacna1e*) in *Paxip*^{Δ/Δ} B cells displayed more than a fourfold decrease in H3K4me3 compared to controls, and 10 regions displayed more than a threefold decrease in H3K4me3 (Fig. 1A and fig. S3, A and B). No regions displayed consistently increased H3K4me3 in *Paxip*^{Δ/Δ} B cells (fig. S3A). Consistent with PTIP deficiency in *Paxip*^{Δ/Δ} cells, the *Paxip* gene itself also displayed reduced H3K4me3 (Fig. 1A) because of Cre-mediated deletion of its first exon (3). Taken together, our data demonstrate a selective role for PTIP in promoting histone methylation in activated B cells.

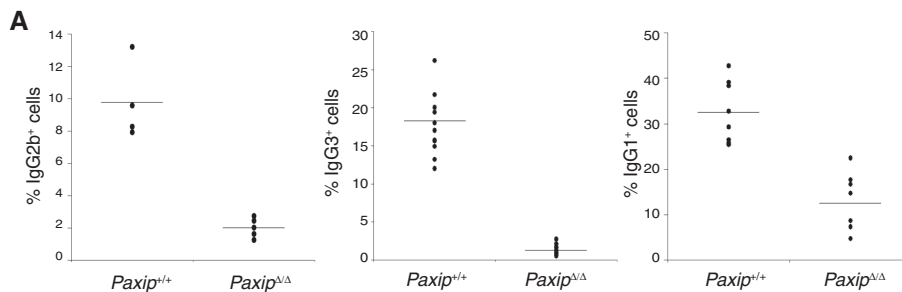
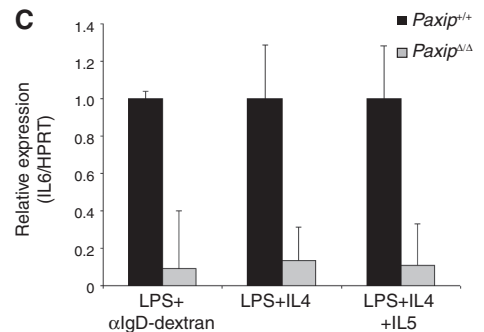
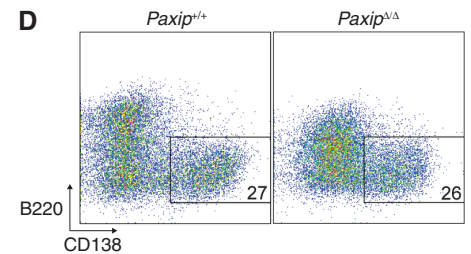
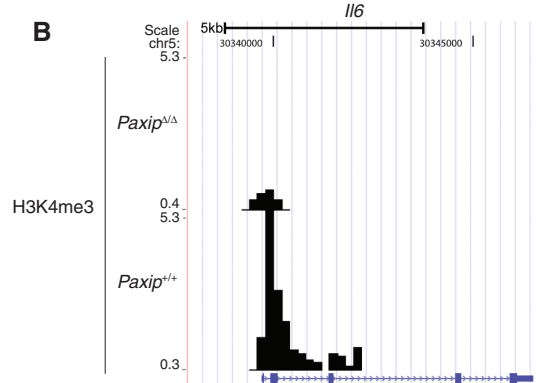


Fig. 2. Ig CSR defects in *Paxip*^{Δ/Δ} B cells. (A) Average percentage of IgG⁺ B cells from flow cytometric analyses of *ex vivo* stimulation (IgG2b⁺ and IgG3⁺ from LPS and α-IgD-dextran and IgG1⁺ from LPS and IL-4). Each dot represents an individual mouse, and the line represents the average (IgG2b⁺: 4.8-fold, *P* = 0.006; IgG3⁺: 14.1-fold, *P* = 0.00000005; IgG1⁺: 2.6-fold, *P* = 0.00003). (B) H3K4me3 ChIP-Seq profiles at the *Il6* gene in B cells stimulated with LPS and α-IgD-dextran for 2 days. A UCSC (University of California Santa Cruz) gene annotation for *Il6* is shown at the bottom. Data are representative of three independent experiments. (C) *Paxip*^{Δ/Δ}-activated B cells display decreased *Il6* transcript amounts. RT-qPCR analysis of *Il6* transcripts from B cells stimulated for 3 days under the indicated conditions. (D) Flow cytometric analysis of B



cells stimulated with LPS, IL-4, and IL-5 for 5 days and stained with anti-CD138 as a marker for plasma cells. Numbers indicate the percentage of total live CD138⁺ B220^{low} B cells. Data are representative of two independent experiments.

The *Igh*- $\gamma 2b$ and *Igh*- $\gamma 3$ switch regions showed the greatest decrease in H3K4me3 from LPS-induced *Paxip*^{Δ/Δ} B cells (Fig. 1, A and B). Moreover, *Paxip*^{Δ/Δ} and control B cells stimulated with LPS and IL-4 displayed PTIP-dependent H3K4me3 at *Igh*- $\gamma 1$ (fig. S4), indicating that PTIP also promotes H3K4me3 at other *Igh* switch regions under different conditions of B cell activation. No-

tably, the PTIP-dependent H3K4me3 at activated *Igh* switch regions occurs independently of AID-induced DNA damage (fig. S4), consistent with H3K4me3 associating with transcription rather than with DNA DSBs (14). PTIP deficiency, however, had no effect on H3K4me3 marking the *Igh*- μ enhancer (5'E μ), μ switch repeat region (S μ), or *Igh*- ϵ switch repeat region (S ϵ) under either

stimulation condition (Fig. 1B and fig. S4), demonstrating the specificity of PTIP-mediated histone methylation. Normal H3K4me3 across the 5'E μ region is consistent with the normal surface IgM expression observed on *Paxip*^{Δ/Δ} B cells (fig. S2D).

Ig class-switching defects in *Paxip*^{Δ/Δ} B cells. Aberrant regulation of *Igh* switch regions and

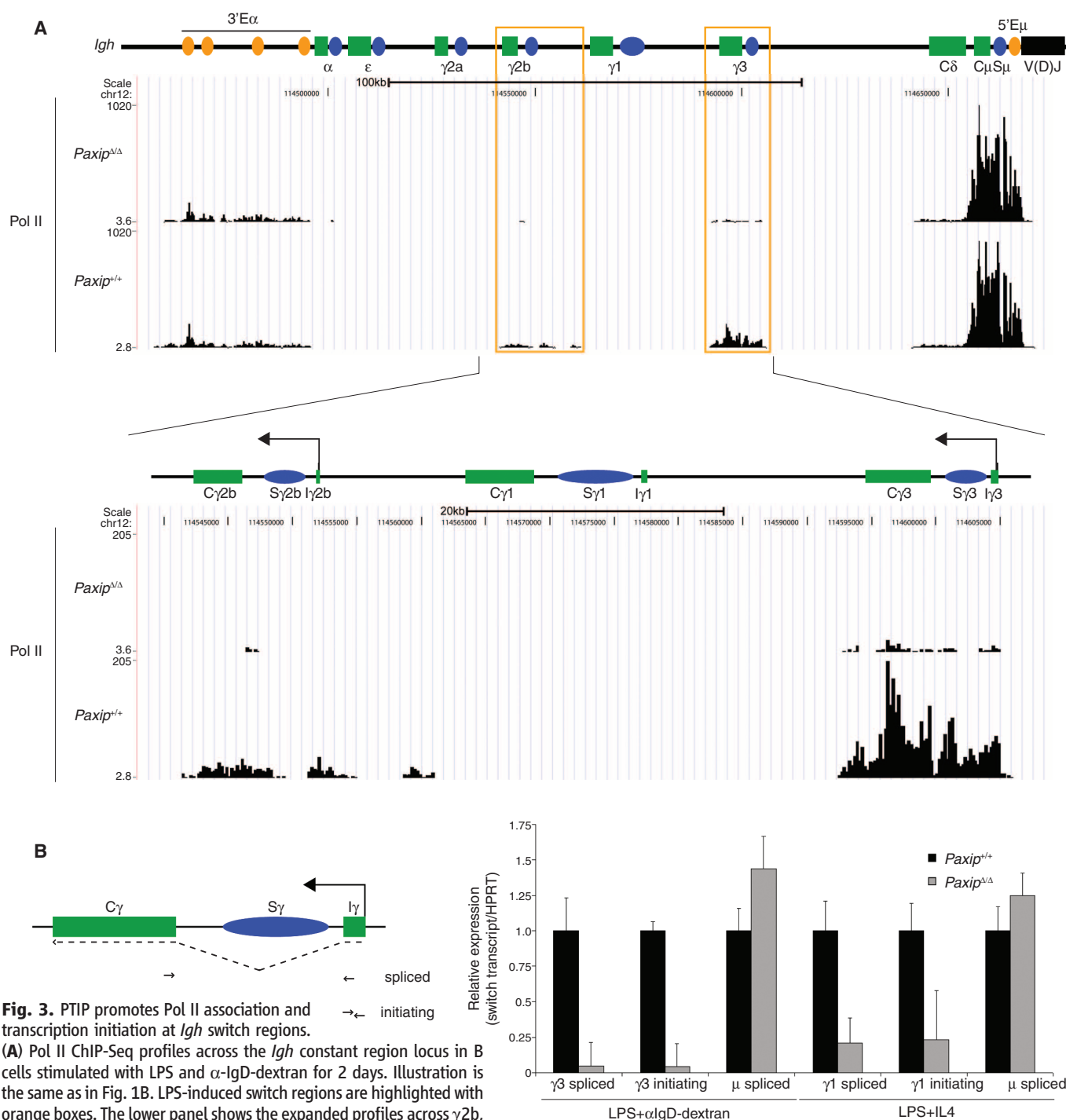


Fig. 3. PTIP promotes Pol II association and transcription initiation at *Igh* switch regions. (A) Pol II ChIP-Seq profiles across the *Igh* constant region locus in B cells stimulated with LPS and α -IgD-dextran for 2 days. Illustration is the same as in Fig. 1B. LPS-induced switch regions are highlighted with orange boxes. The lower panel shows the expanded profiles across $\gamma 2b$, $\gamma 1$, and $\gamma 3$. Black arrows indicate sites of LPS- and α -IgD-dextran-induced transcription at $\gamma 3$ and $\gamma 2b$ switch regions. "I" indicates initiating exon segment. (B) Left, illustration showing location of oligonucleotides used in RT-qPCR. Right, RT-qPCR analysis of spliced and initiating *Igh* switch transcripts from B cells stimulated under the indicated conditions for 3 days. Data are representative of two independent experiments.

Il6 have well-established implications for mature B cell function (18, 23). To understand the physiological relevance of PTIP-dependent H3K4me3 at *Igh* switch regions, we examined Ig class switching. Upon LPS stimulation, *Paxip*^{Δ/Δ} B cells displayed 14- and 4.8-fold decreases in the frequency of IgG3 and IgG2b switching, respectively (Fig. 2A and fig. S5, A and B). *Paxip*^{Δ/Δ} B cells also displayed a 2.6-fold decrease in IgG1 switching upon LPS and IL-4 stimulation (Fig. 2A and fig. S5, A and B). When CSR was assayed directly from genomic DNA of B cells stimulated with LPS and IL-4, *Paxip*^{Δ/Δ} B cells displayed a similar defect in the level of Sμ-Sγ1 switch junctions (fig. S5C). As expected,

Paxip^{Δ/Δ} B cells showed normal cell proliferation (figs. S2E and S5, A and B) and cell survival (fig. S5D). We conclude that PTIP is critical for IgG3, IgG2b, and to a lesser extent, IgG1 class switching.

IL-6 was further analyzed because it can function in both antibody secretion and the development and tumorigenesis of plasma cells (23) (Fig. 1A). We found that H3K4me3 at *Il6* was both LPS inducible and PTIP dependent (fig. S1C and Fig. 2B) and that mRNA expression of *Il6* was also impaired in *Paxip*^{Δ/Δ} B cells (Fig. 2C). These defects, however, had no apparent effect on plasma cell differentiation ex vivo, as monitored by CD138 surface expression (Fig.

2D). Moreover, the IgG3 class-switching defect in *Paxip*^{Δ/Δ} B cells was not rescued with exogenous recombinant IL-6 in the culture medium (fig. S6).

PTIP promotes Ig switch region transcription initiation. For most actively transcribed genes, H3K4me3 and Pol II accumulate within 2 kb of the transcription start sites (24). In contrast, H3K4me3 peaks at *Igh* switch regions are more broad, spanning up to 7 kb downstream of the germline transcript promoter, and include the mutagenic switch repeats and downstream of the switch region (Fig. 1B and figs. S4 and S7, A and B) (25). This broad H3K4me3 distribution at switch regions correlates with ob-

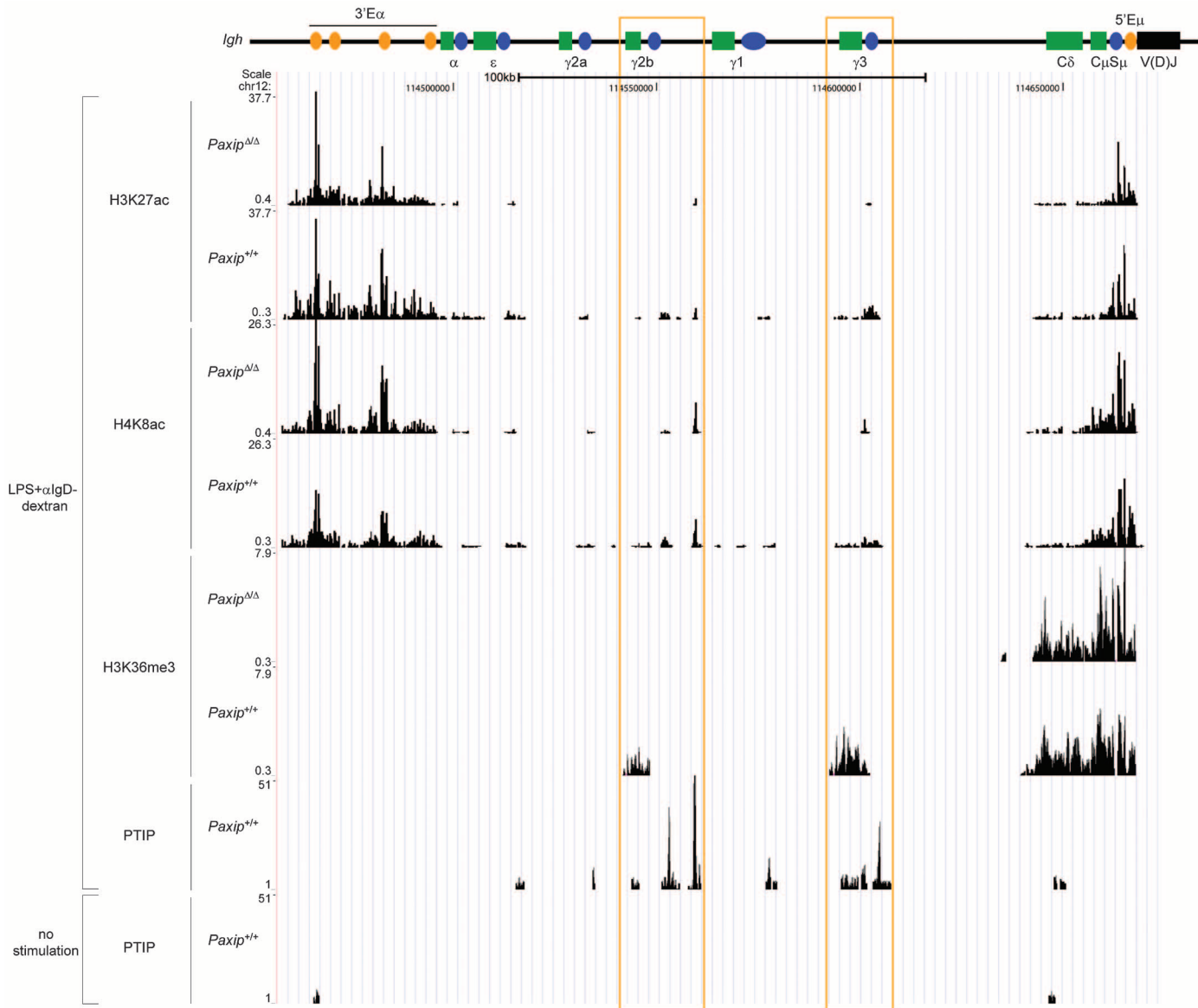


Fig. 4. PTIP directly localizes to *Igh* upon LPS stimulation and promotes histone acetylation and H3K36me3 at activated *Igh* switch regions. The indicated histone modification (H3K27ac, H4K8ac, and H3K36me3) and PTIP ChIP-Seq profiles across the *Igh* constant region locus in B cells either stimulated with LPS and α-IgD-dextran for 2 days or with no stimulation.

Significant PTIP association is presented as fold change enrichment of control/ *Paxip*^{Δ/Δ} island tag counts. PTIP ChIP-Seq data in stimulated cells are representative of two independent experiments. Illustration is the same as in Fig. 1B. LPS-induced switch regions are highlighted with orange boxes.

served accumulation of Pol II at switch regions (Fig. 3A) (25, 26). Therefore, we considered whether PTIP might be important for elongation or splicing of switch transcripts. To investigate whether PTIP regulates transcription of *Igh* switch regions, we measured germline switch

transcripts that had been spliced from the initiating (I) exon located 5' of the switch region to the constant (C) exon located 3' of the switch region from stimulated *Paxip*^{Δ/Δ} and control B cells (Fig. 3B). IgM-expressing B cells express a μ sterile transcript, and we found that

Paxip^{Δ/Δ} B cells displayed near-normal μ switch transcript amounts in both conditions (Fig. 3B). This is consistent with the normal H3K4me3 patterns at E μ and S μ in the absence of PTIP (Fig. 1B and fig. S4). In contrast, both γ 3 and γ 1 spliced switch transcripts, important for

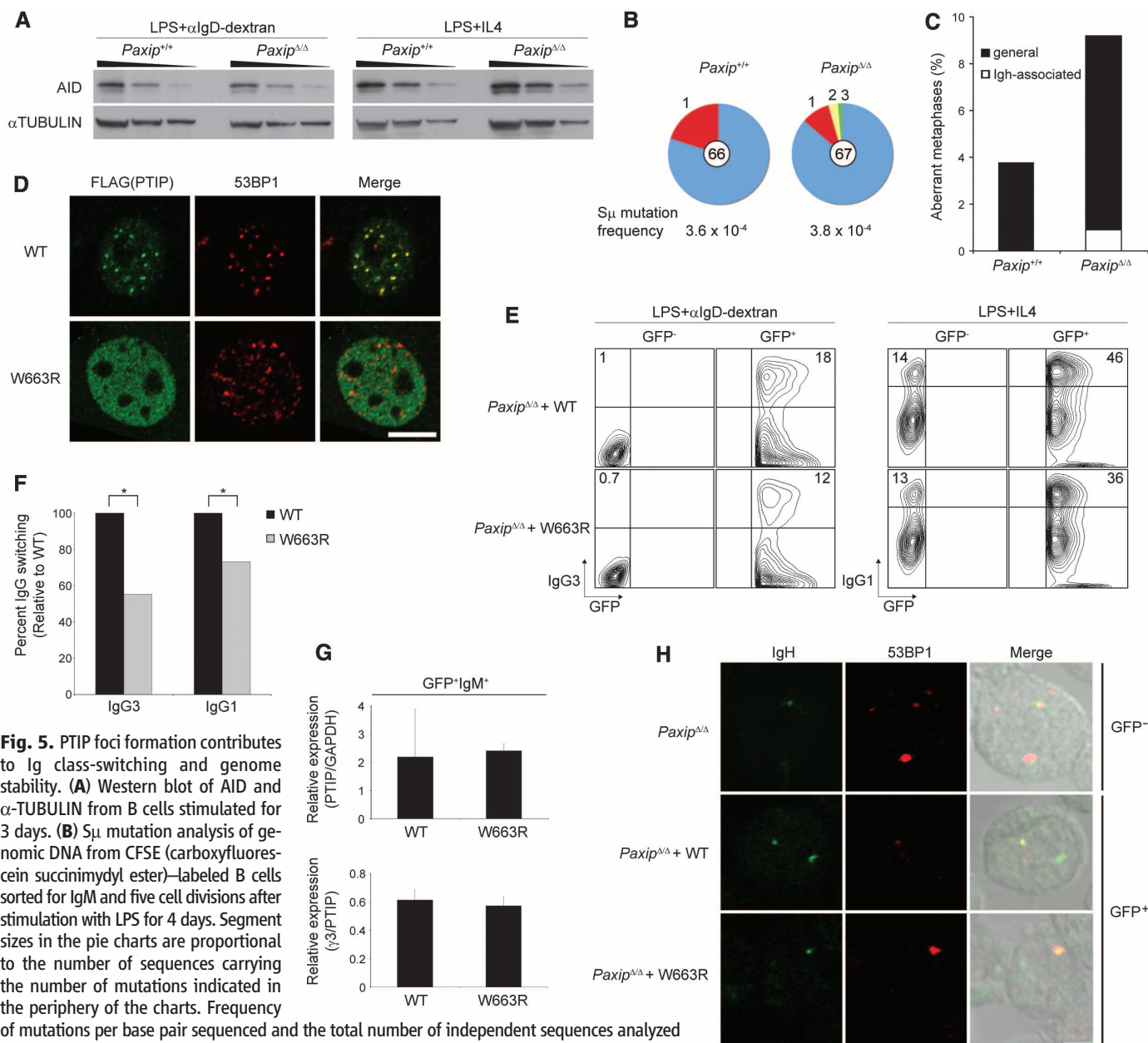


Fig. 5. PTIP foci formation contributes to Ig class-switching and genome stability. (A) Western blot of AID and α -TUBULIN from B cells stimulated for 3 days. (B) S μ mutation analysis of genomic DNA from CFSE (carboxyfluorescein succinimide ester)-labeled B cells sorted for IgM and five cell divisions after stimulation with LPS for 4 days. Segment sizes in the pie charts are proportional to the number of sequences carrying the number of mutations indicated in the periphery of the charts. Frequency of mutations per base pair sequenced and the total number of independent sequences analyzed are indicated below and in the center of each chart, respectively. Data are representative of two independent experiments. (C) Genomic instability analysis of metaphase spreads from B cells stimulated with LPS for 3 days (two mice of each genotype, *Paxip*^{+/+}; *n* = 261; *Paxip*^{Δ/Δ}; *n* = 218). Abnormalities specifically associated with the *Igh* locus (*Igh*-associated) or with all other chromosomes (general) are shown. (D) Immunofluorescent images of retrovirally infected MEFs exposed to 10-Gy irradiation with 4 hours recovery showing PTIP and 53BP1 foci formation. Bar, 20 μ m. (E) Flow cytometric analysis of retrovirally infected *Paxip*^{Δ/Δ} stimulated B cells stained 3 days after infection with IgG antibodies. Numbers indicate the percentages of total live IgG⁺ cells in GFP⁻ (no retroviral expression) or GFP⁺ (retroviral expression) gates. (F) Average percentage of IgG switching from infection experiments. Data are from four independent experiments for each condition (*P < 0.05). (G) RT-qPCR of *Paxip* (top) and *Igh*- γ 3 switch (bottom) transcripts from retrovirally infected GFP⁺IgM⁺ sorted *Paxip*^{Δ/Δ} B cells stimulated with LPS. (H) Representative images of sorted B cells stimulated with LPS and IL-4 for 2 days and stained with α 53BP1 antibody (red) before FISH detection of the *Igh*-C α region (green). Among the total number of cells examined, those that contained 53BP1 and *Igh*-C α foci were analyzed [GFP⁻ *Paxip*^{Δ/Δ}, 100 cells; wild-type (WT) control GFP⁺, 89 cells; W663R mutant GFP⁺, 97 cells]. Coincidence of a 53BP1 focus with one or both *Igh*-C α alleles was detected in 42% of GFP⁻ *Paxip*^{Δ/Δ}, 30% of WT control GFP⁺, and 43% of W663R mutant GFP⁺ cells. Bar, 5 μ m.

IgG CSR, were severely impaired in *Paxip*^{ΔΔ} B cells under their respective stimulation conditions (Fig. 3B). Spliced transcripts at *Igh*-ε were unchanged in *Paxip*^{ΔΔ} B cells (fig. S7C), consistent with no change in H3K4me3 at this switch region in the absence of PTIP (fig. S4).

To further delineate at which stage PTIP functions in promoting *Igh*-γ germline switch transcript expression, we also assayed for unspliced and initiating switch transcripts. *Paxip*^{ΔΔ} B cells showed similar deficiencies both in unspliced (fig. S7D) as well as in initiating switch transcripts (Fig. 3B) as were observed for spliced switch transcripts when we assayed across the IγCγ exon junction (Fig. 3B). We conclude that PTIP functions at a stage preceding pre-mRNA splicing to promote initiating *Igh* switch transcript expression.

In yeast, evidence suggests that initiating Pol II recruits the H3K4 methylase activity to mark transcription start sites (14). To test whether PTIP promotes *Igh* switch region transcripts at the level of Pol II association or downstream of pre-initiation complex assembly, we performed Pol II ChIP-Seq on *Paxip*^{ΔΔ} and control B cells stimulated with LPS. At *Igh*, although no major changes were observed at the μ, δ, or 3' α enhancer (Eα) regions, Pol II association was severely impaired at both *Igh*-γ2b and γ3 regions (Fig. 3A). Furthermore, at the other sites displaying PTIP-dependent H3K4me3, including *Il6* and *Paxip* itself, Pol II association was also impaired (fig. S8). We conclude that, upon LPS stimulation, PTIP is required for Pol II association at downstream switch regions along with a subset of the other regions displaying PTIP-dependent H3K4me3.

Histone acetylation facilitates decondensation of chromatin, which may promote transcriptional activation by increasing the accessibility of transcription factors and Pol II to promoters (27). To better understand the mechanism for PTIP-dependent Pol II association at *Igh* switch regions and other PTIP-affected regions, we performed ChIP-Seq for a number of histone acetylation marks (H2BK5ac, H3K9ac, H3K27ac, and H4K8ac) along with the H3K36me3 mark associated with transcription elongation (24). At *Igh*, our data indicate that PTIP is required for all of these histone modifications at activated switch regions, and, similar to H3K4me3 and Pol II, PTIP is dispensable for these modifications at the μ, δ, and 3'Eα regions (Fig. 4 and fig. S9). Thus, loss of H3K4me3 correlates with impaired histone acetylation. Although PTIP is also required for H3K4me2 at activated *Igh* switch regions (fig. S9), the H3K4me1 mark was present similarly across the *Igh* locus in both *Paxip*^{ΔΔ} and control B cells (fig. S9), indicating that PTIP is required for most but not all chromatin signatures of *Igh* switch region transcription. This PTIP dependency for other histone modifications largely holds true for all regions displaying PTIP-dependent H3K4me3 (fig. S8). Taken together, our results indicate that loss of PTIP correlates with impaired H3K4me2/3,

histone acetylation, and Pol II association and suggest that chromatin accessibility at *Igh* switch regions may be regulated by a hierarchy of histone modifications.

To test whether PTIP-dependent H3K4me3 in activated B cells is directly mediated by the PTIP-associated methyltransferase complex, we performed genome-wide association analysis of PTIP and the shared MLL-like complex component, ASH2, using ChIP-Seq. Substantial enrichment of PTIP was observed at 9647 islands in LPS-stimulated control B cells, and localization was substantially enriched at promoter regions (fig. S10, A and B). By comparison, ASH2 binding in LPS-stimulated B cells was observed at 27,806 islands and was also enriched at promoter regions (fig. S10, A and B). Although the majority (78.6%) of LPS-induced H3K4me3 that we identified earlier (Fig. 1A and table S1) also displayed ASH2 binding (fig. S10C), only 27.8% show direct PTIP binding (fig. S10C), suggesting that PTIP function in gene expression during B cell activation may be very limited. Notably, nearly all (94.7%) of the LPS-induced H3K4me3 sites showing PTIP binding also displayed ASH2 binding (fig. S10C). These data are consistent with PTIP associating with an MLL-like complex, which colocalizes with H3K4me3 marks at transcription start sites (1–4). These data also suggest that PTIP may be important for targeting the MLL3-MLL4 complex to a subset of genomic loci. Indeed, substantial PTIP and ASH2 ChIP-Seq colocalization was observed at five of the six regions displaying PTIP-dependent H3K4me3, including *Igh*-γ3, *Igh*-γ2b, *Il6*, *Slit3*, and *Paxip* itself (Fig. 4 and figs. S8 and S11A).

By comparing resting and stimulated B cells, we also found that PTIP and ASH2 localization at *Igh* switch regions was dependent on LPS stimulation (Fig. 4 and fig. S11A), suggesting that the PTIP-associated complex is actively recruited to the *Igh* locus during B cell activation. Consistent with these data, localization of PTIP and another shared MLL-like complex component, RBBP5, was also observed near the *Igh*-γ1 transcription start site in LPS- and IL-4-stimulated B cells using ChIP-qPCR (fig. S11B). Furthermore, RBBP5 localization at *Igh*-γ1 was dependent on PTIP (fig. S11B), suggesting that PTIP is required for targeting the methyltransferase complex to *Igh* switch regions. As a specificity control, we did not observe enrichment of PTIP by ChIP-qPCR (quantitative polymerase chain reaction) near the *Igh*-γ1 transcription start site in LPS- and IL-4-stimulated mouse embryonic fibroblasts (MEFs) (fig. S11C). We conclude that PTIP-dependent H3K4me3 at *Igh*-γ3, *Igh*-γ2b, and *Igh*-γ1 in stimulated B cells results from direct association of the PTIP-associated H3K4 methyltransferase complex nearby their transcription start sites.

PTIP accumulation at DNA breaks contributes to CSR independently of its role in transcription. AID expression is required for mutations at *Igh* switch regions and for CSR (28–31); however, AID protein expression was similar in *Paxip*^{ΔΔ}

and control B cells (Fig. 5A), consistent with normal H3K4me3 and Pol II at the *Aicda* locus in *Paxip*^{ΔΔ} B cells (fig. S1E). Similarly, we observed no major differences in H3K4me3, Pol II association, or expression of genes implicated in CSR, suggesting that disruption of *Paxip* does not cause indirect expression defects in known CSR-related factors (fig. S12, A and B). Moreover, a similar frequency of mutations was observed in *Paxip*^{ΔΔ} B cells in a region immediately 5' of the Sμ repeat sequence (Fig. 5B), suggesting that AID targeting and mutation at Sμ occurs independently of PTIP.

PTIP-mediated changes in *Igh* chromatin structure could also affect NHEJ of broken switch regions. To directly assay for alterations in DNA repair, we analyzed switch recombination junctions from successfully class-switched IgG1-positive B cells. We found that Sμ-Sγ1 junctions were indistinguishable between *Paxip*^{ΔΔ} and control, showing no differences in the amount of donor/acceptor homology at the junctions, suggesting that classical NHEJ is proficient in *Paxip*^{ΔΔ} B cells (fig. S13). Nevertheless, B cells with CSR-related DNA repair deficiencies show *Igh*-associated DNA breaks and general genomic instability (21, 32, 33). Indeed, we found that 8.3% of metaphases from *Paxip*^{ΔΔ} B cells showed general genomic instability outside of the *Igh* locus, compared to 3.8% in control cells, marked by chromosome breaks, chromatid breaks, and translocations (Fig. 5C). Furthermore, 0.9% of *Paxip*^{ΔΔ} metaphases displayed *Igh*-associated instability compared with none in control cells (Fig. 5C). We conclude that PTIP suppresses both general and *Igh*-associated genomic instability in B cells. DNA breaks and translocations at *Igh* may arise from AID-induced mutations at the μ, γ3, and γ2b regions because these are the transcriptionally active switch loci upon LPS stimulation (17, 18, 21). Given that AID is targeted to the upstream Sμ region (Fig. 5B) while transcription (and presumably accessibility) of downstream (Sγ) switch regions is impaired (Fig. 3, A and B), we presume that the observed *Igh* instability in PTIP-deficient cells is the result of unresolved AID-dependent breaks at Sμ.

53BP1 accumulates at sites of AID-induced *Igh* breaks and promotes DNA end-joining during CSR (21). We further examined a role for PTIP in DNA repair of CSR-associated breaks because PTIP contains six BRCT domains and can physically interact with 53BP1. A mutation of Trp⁶⁶³ to Arg (W663R) within BRCT domain 3 (BRCT3) of PTIP abolishes irradiation-induced foci formation of PTIP (8, 11), demonstrating the critical importance of this residue in targeting PTIP to DNA DSBs. To test whether accumulation of PTIP at sites of AID-induced DNA damage plays a role in Ig class switching, we generated retroviral expression constructs for B cell infection containing either control or W663R mutant PTIP and IRES-GFP (green fluorescent protein) to label the infected cells. After verifying that our ectopically expressed

PTIP protein accumulated at sites of DSBs and the W663R mutant retrovirus expressed a DNA damage foci-defective PTIP mutant (Fig. 5D), we infected stimulated *Paxip*^{ΔΔ} B cells with retrovirus expressing either wild-type control or W663R mutant and measured class switching. First, comparing switching in GFP⁺ and GFP⁻ cells infected with control retrovirus indicated that both IgG3 and IgG1 class-switching defects observed in *Paxip*^{ΔΔ} B cells are fully rescued (Fig. 5E), demonstrating that ectopic PTIP expression is functional. In contrast, the W663R mutant retrovirus restored IgG3 and IgG1 class switching only to 52% and 66%, respectively, of switching restored by control retrovirus (Fig. 5, E and F). This difference in the efficiency of rescue is not caused by impaired PTIP or γ 3 spliced switch transcript amounts (Fig. 5G). These data indicate that the defect in class switching observed in W663R mutant B cells, unlike that in *Paxip*^{ΔΔ} B cells, is not due to aberrant germline switch transcription.

Mutation of W663 or any of the four C-terminal BRCT domains abolishes IR-induced 53BP1 coprecipitation with PTIP (8, 11). To determine whether disruption of *Paxip* or W663 impairs 53BP1 accumulation at *Igh* in B cells undergoing CSR, we infected *Paxip*^{ΔΔ} B cells with either W663R mutant or control retrovirus and processed the cells for immunocytochemistry–fluorescence in situ hybridization (FISH) to visualize protein and DNA simultaneously (30). W663R mutant and control B cells showed a similar percentage of 53BP1/*Igh* colocalizing (Fig. 5H), suggesting that defective CSR in the W663R mutant was not caused by impaired 53BP1 accumulation at *Igh*. These data support our observations (fig. S14) and those of others (11) that PTIP is dispensable for IR-induced 53BP1 foci formation in MEFs.

Discussion. Observations from mice with *Igh* switch promoter deletions and subsequent biochemical studies (17–20) have together demonstrated that switch-region transcription targets AID activity to *Igh* and is required for AID-dependent DSB formation and class switching (fig. S15, step 3). Histone modifications at the *Igh* locus and interactions with the transcription machinery have been suggested to be important for AID accessibility and targeting (25, 34, 35), but there has been little genetic evidence to support this notion. Our experiments call attention to PTIP as a critical factor for directly mediating histone modifications and promoting transcription initiation of germline *Igh* switch regions to target AID activity and also functioning, subsequently, in the repair of AID-induced DNA breaks.

PTIP promotes *Igh* switch transcription initiation. We propose that the downstream *Igh* constant region locus, as a cluster of gene segments that lack H3K4me3, Pol II, and transcription initiation, is an example of a tightly repressed gene cluster (15) that, upon B cell stimulation, requires PTIP to alleviate regulatory mechanisms

that ensure proper targeting and stability of the switch regions (36). Because disruption of PTIP does not appear to alter MLL3-MLL4 complex integrity (2), our data suggest that PTIP confers specificity for recruitment of MLL3-MLL4 activity to particular genomic regions. Based on the observations that PTIP can directly interact with the PAX2 transcription factor (37) and mediate recruitment of H3K4me activity to a PAX2-dependent promoter (3), we speculate that PTIP-mediated H3K4me results from direct interaction with DNA-binding transcription factors (fig. S15, step 1) (36). In this model, upon PTIP-associated MLL3-MLL4 complex recruitment, H3K4me3 is catalyzed at transcription start sites (fig. S15, step 2). The H3K4me3 mark could then recruit acetylases and chromatin remodelers that alter chromatin structure at transcription start sites, promoting recruitment and/or stabilization of Pol II to initiate transcription (fig. S15, step 2). Nevertheless, we cannot exclude an alternative model in which PTIP stabilizes a transcription factor–DNA complex at the *Igh* locus leading to more efficient transcription initiation by Pol II, which in turn promotes H3K4 di- and trimethylation.

PTIP functions in DNA repair during class switching. γ -H2AX amplifies the DNA damage signal, in part, by accumulating the E3 ubiquitin ligase RNF8 to promote H2A/H2AX ubiquitination critical for subsequent accumulation of PTIP to DSBs (9, 11). Because CSR-associated DSBs are marked by γ -H2AX foci (30) and both H2AX- and RNF8-deficient mice also exhibit mild defects in CSR due to DSB repair defects (38–40), we speculate that, after AID-dependent DSB formation (fig. S15, step 3), PTIP may act downstream of H2AX and RNF8 to facilitate synapsis and/or repair efficiency of CSR-associated DNA breaks (fig. S15, step 4). Although DNA damage–induced accumulation of PTIP promotes efficient Ig class switching, this DNA repair function, however, cannot entirely account for the severe class-switching defects observed in *Paxip*^{ΔΔ} B cells, which arise to a larger extent from impaired transcription initiation of downstream *Igh* switch regions.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1187942/DC1
Materials and Methods
Figs. S1 to S16
Tables S1 and S2
References

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Cosmological Constraints from Strong Gravitational Lensing in Clusters of Galaxies

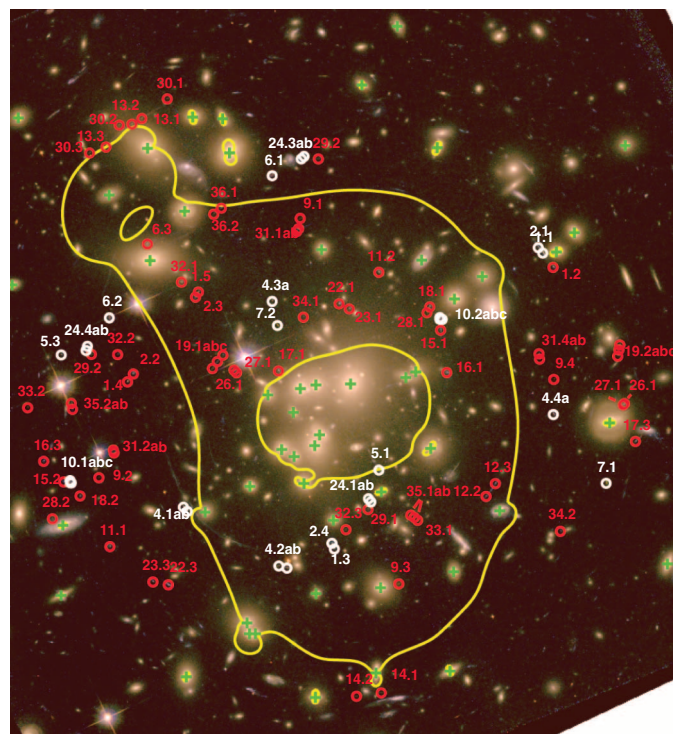
Eric Jullo,^{1,2} Priyamvada Natarajan,^{3,4*} Jean-Paul Kneib,² Anson D'Aloisio,⁴ Marceau Limousin,^{2,5} Johan Richard,⁶ Carlo Schmid²

Current efforts in observational cosmology are focused on characterizing the mass-energy content of the universe. We present results from a geometric test based on strong lensing in galaxy clusters. Based on Hubble Space Telescope images and extensive ground-based spectroscopic follow-up of the massive galaxy cluster Abell 1689, we used a parametric model to simultaneously constrain the cluster mass distribution and dark energy equation of state. Combining our cosmological constraints with those from x-ray clusters and the Wilkinson Microwave Anisotropy Probe 5-year data gives $\Omega_m = 0.25 \pm 0.05$ and $w_x = -0.97 \pm 0.07$, which are consistent with results from other methods. Inclusion of our method with all other available techniques brings down the current 2σ contours on the dark energy equation-of-state parameter w_x by $\sim 30\%$.

Measurements of the Hubble diagram for type Ia supernovae (1–4) combined with constraints from the Wilkinson Microwave Anisotropy Probe (WMAP5) (5, 6), cosmic shear observations (7–11), cluster baryon fractions (12), cluster abundances (13), and baryon acoustic oscillations (BAOs) from galaxy surveys (14–16) suggest that $\sim 72\%$ of the total energy density of the universe is in the form of an unknown constituent with negative pressure—the so-called dark energy that powers the measured accelerating expansion. These observations probe the equation-of-state parameter w_x , defined as the ratio of pressure to energy density, through its effect on the expansion history and structure of the universe. The current goal of cosmology is to understand the properties of dark energy by placing tighter constraints on its equation of state. In the currently favored flat Λ CDM (Lambda Cold Dark Matter) model (17), dark energy is attributed to a cosmological constant for which $w_x = -1$. Type Ia supernovae, BAOs, cluster abundances, and cosmic shear appear to be very promising techniques to tighten constraints on the equation-of-state parameter in the near future. Because all of these techniques have biases, systematics, and degeneracies, it is only in combination that robust estimates of cosmological parameters can be obtained.

In this work, we present results from a technique that exploits the strong gravitational lensing of distant background galaxies by massive galaxy clusters. Through their effect on the local space-time geometry, massive foreground structures cause the deflection and shearing of light rays originating from distant sources. In the case of strong lensing, the light beams are deflected so strongly that they can often result in the observation of several distorted images of a given single background galaxy. The positions of these multiple images depend strongly on the detailed properties of the lens mass distribution (18–20).

Fig. 1. The critical lines for a source at $z = 3$ are overplotted in yellow on the HST ACS image of Abell 1689. The lensing mass model used is the one from which we derived cosmological constraints. In addition to two large-scale clumps and the BCG, this model includes the contribution of 58 cluster galaxies. The positions of cluster galaxies are marked with green crosses. Overplotted in white are the 28 multiple images arising from 12 families that we used in this work; the red circles mark the positions of the rejected images.



¹Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA. ²Laboratoire d'Astrophysique de Marseille, CNRS, Université de Provence, 38 rue Frederic Joliot-Curie, 13388 Marseille Cedex 13, France. ³Department of Astronomy, Yale University, Post Office Box 208101, New Haven, CT 06511-8101, USA. ⁴Department of Physics, Yale University, Post Office Box 208120, New Haven, CT 06520-8120, USA. ⁵Dark Cosmology Centre, Niels Bohr Institute, University of Copenhagen, Juliane Maries Vej 30, 2100 Copenhagen, Denmark. ⁶Institute for Computational Cosmology, Department of Physics, Durham University, South Road, Durham DH1 3LE, UK.

*To whom correspondence should be addressed. E-mail: priyamvada.natarajan@yale.edu

Because the image positions also depend on the angular diameter distance ratios between the lens, source, and observer, they encapsulate information about the underlying cosmology. We capitalize on this dependence on the geometry to derive constraints on the cosmological parameters Ω_m (the mean matter density) and w_x .

Constraining the energy content of the universe using multiple sets of arcs in cluster lenses has been explored in the past (21–27). In particular, simultaneous inversion of the lens and derivation of cosmological constraints can be performed based on the cosmological sensitivity of the angular size–redshift relation with sources at distinct redshifts (22). In this method, the angular diameter distance ratios for two images from different sources defines the “family ratio” Ξ , from the cosmological dependence of which constraints on Ω_m and w_x are extracted

$$\Xi(z_1, z_{s1}, z_{s2}; \Omega_m, \Omega_X, w_x) = \frac{D(z_1, z_{s1}) D(0, z_{s2})}{D(0, z_{s1}) D(z_1, z_{s2})} \quad (1)$$

where z_1 is the lens redshift, z_{s1} and z_{s2} are the two source redshifts, and $D(z_1, z_2)$ is the angular diameter distance.

Application of this method to the cluster Abell 2218 using four multiple image systems at distinct redshifts resulted in $\Omega_m < 0.37$ and $w_x < -0.80$ for a flat universe (26). A recent feasibility study demonstrates that the degeneracies of this technique are entirely distinct from those of other cluster methods and that combining the results from several simulated clusters with >10 multiple

image families in each can provide a powerful probe of dark energy (27).

We have applied this technique to the massive lensing cluster Abell 1689 at redshift $z = 0.184$. Based on images from the Advanced Camera for Surveys (ACS) aboard the Hubble Space Telescope (HST), this cluster has 114 multiple images from 34 unique background galaxies, 24 of which have secure spectroscopic redshifts (ranging from $z \sim 1$ to 5), obtained with the Very Large Telescope (VLT) and Keck Telescope spectrographs (28, 29). Abell 1689 is among the richest clusters in terms of the number density of galaxies in its core. It is also among the most luminous of galaxy clusters in x-ray wavelengths, with an absolute x-ray luminosity of $L_X = 20.74 \times 10^{37}$ W (30). Observationally, Abell 1689 consists of two groups of galaxies: a dominant one located at the center coincident with the peak of x-ray emission, and a secondary concentration about 1 arcminute northeast of the main one. Studies have shown that this second northern group is at a slightly higher redshift, thus suggesting that these two groups might actually be merging (31). The projected mass enhancement produced by such a merging configuration could therefore explain the stunningly large number of multiple images identified in this cluster. Previous work shows that the mass distribution of Abell 1689 is well modeled with a set of parameterized elliptical pseudo-isothermal lensing potentials (29). We used the most recent parametric model of Abell 1689, which is able to reproduce the observed image configurations to within an average positional accuracy of 2.87 arcseconds, assuming a Λ CDM cosmology. We solved the lens equation in the source plane for Abell 1689 because it is computationally effi-

cient. Inverting in the lens (image) plane provides additional information but is computationally prohibitive at present (29). Our simplified model that has a total of 21 free parameters consists of two large-scale potentials and a galaxy-scale potential for the central brightest cluster galaxy (BCG), and includes the modeling of 58 of the brightest cluster galaxies. Therefore, we explicitly include the effect of substructure in the lens plane and assigned potentials associated with bright cluster galaxies. The velocity dispersion and scale radii of all but one (the BCG) of the cluster galaxies were assumed to follow empirically motivated scaling relations, which have been previously used to model cluster lenses (32, 33).

Despite the large number of multiple images observed, not all of them can be used to constrain cosmology. From the initial 114 images, we (i) used only those with robust, measured spectroscopic redshifts and (ii) excluded those in the regions of the cluster with low signal-to-noise ratio in the mass reconstruction. This selection results in the culling of multiple images that lie in the most uncertain regions of the mass distribution. Moreover, we identified several bright spots in some well-resolved multiple images, which we used to increase the number of families. Applying criterion (i) resulted in a catalog of 102 images. Imposing criteria (i) and (ii), we obtained a catalog of 28 images arising from 12 families, all with measured spectroscopic redshifts (Fig. 1), providing a total of 32 constraints. We assumed flatness as a prior ($\Omega_{\text{tot}} = 1$) and fixed the Hubble parameter at $H_0 = 74$ km/s/Mpc (34), because our cosmography test is not sensitive to the value of the Hubble parameter.

For each of the observed image systems with n images, we determined the goodness of fit for

a particular set of model parameters using a source plane χ^2

$$\chi^2 = \sum_{i=1}^n \frac{\left[M \left(\vec{\beta}_i - \langle \vec{\beta} \rangle \right) \right]^2}{\sigma_i^2} \quad (2)$$

where $\vec{\beta}_i$ is the source-plane position corresponding to image i , $\langle \vec{\beta} \rangle$ is the family barycenter, M is the magnification tensor, and σ_i is the total (observational and modeling) error. The total χ^2 was obtained by summing over families and was used in conjunction with a Markov Chain Monte Carlo (MCMC) sampler to probe the posterior probability density function (PDF) as a function of all relevant model parameters (35) [see supporting online material (SOM)]. The key degeneracies with cosmological parameters for this technique arise from the velocity dispersions, ellipticity, and core radii of the large-scale mass clumps in the model (fig. S4).

The angular resolution of HST images is on the order of 0.1 arcseconds. However, the modeling errors are generally larger due to the complexity of the cluster mass distribution, as well as the effect of intervening structures along the line of sight. We quantified the errors due to the presence of structure along the line of sight using the Millennium Simulation halo catalogs (36). We quantified the errors on an image-by-image basis. By randomly slicing through snapshots of the simulation and tiling them at redshift planes between the observer and the source, we constructed 1000 line-of-sight realizations. We then ray-traced through each realization with the Abell 1689 model included to estimate the effect of intervening halos on image positions. In most cases, these line-of-sight halos perturbed image positions but did not alter the multiplicity of the images. These perturbations induce positional displacements on the order of 1 arcsecond. Therefore, about 1 arcsecond of the error between observed image positions and model image predictions can be attributed to the presence of structure along the line of sight behind the cluster. The presence of projected correlated and associated large-scale structure (filaments) increases the cross section to strong gravitational lensing, making these clusters more efficient lenses; however, simulations show that this is a subdominant effect to that of unassociated distant large-scale structure. Therefore, the presence of aligned correlated large-scale structure at the redshift of the cluster does not scupper the recovery of cosmological parameters (37).

Earlier work on reconstructing the mass distribution of several massive lensing clusters shows that the association of dark matter substructures with the locations of the brightest cluster galaxies is well matched by that derived from the Millennium Simulation (33, 38). This supports our use of luminosity-mass scaling relations to map substructure in the cluster.

The second potential source of error arises from modeling uncertainties for the substructure

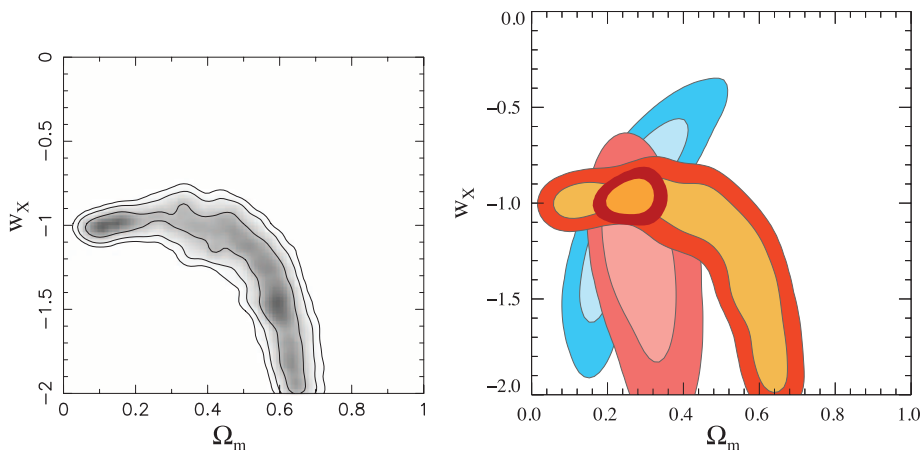


Fig. 2. (Left) The results from the simultaneous Bayesian optimization of the detailed mass distribution and cosmological parameters in the $\Omega_m - w_x$ plane for Abell 1689 using the 28 multiple images belonging to 12 families at distinct redshifts as constraints from strong lensing, including only observational errors. The plotted contours are the 1, 2, and 3σ confidence levels. **(Right)** The results from combining cosmological constraints from WMAP5 + evolution of x-ray clusters + cluster strong lensing (cluster-only methods). The 1 and 2σ contours are plotted: blue contours, constraints from WMAP5; pink contours, x-ray clusters; orange contours, cluster strong lensing. We multiplied the likelihoods for the various techniques to obtain this plot. The degeneracy directions for x-ray clusters and cluster strong lensing are orthogonal.

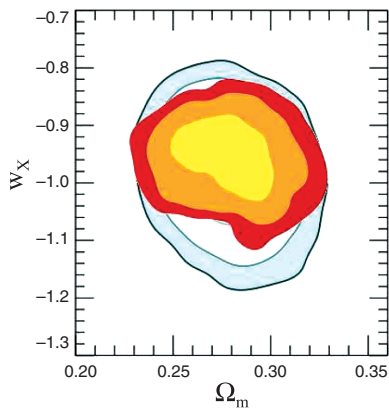


Fig. 3. Combination of constraints from strong lensing, the WMAP5 data (40), the supernovae “Gold sample” (4), SNLS project (41), SNEssence, and the BAO peak from SDSS (16). Contours for non-SL cosmological probes come from the WMAP plotter. The overplotted contours are all 1, 2, and 3σ confidence levels.

in the lens plane. This is likely due to scatter in the assumed scaling relations for the velocity dispersions and scale radii of cluster galaxies. Although the mean correlations between these variables may be well described by simple scaling relations, individual galaxies can deviate substantially from them, introducing errors into the parameter recovery. To quantify these modeling errors, we performed Monte Carlo simulations of the lens system, assuming a 20% scatter in the galaxy scaling relations. This scatter induced modeling errors on the background galaxy image positions that in some cases were as large as ~ 1 arcsecond. Therefore, the estimated errors from substructure effects in the lens plane and along the line of sight are comparable for the selected multiple image families in Abell 1689.

We used the catalog of 102 images (including images with photometric redshift estimates) and our estimates of the observational and modeling errors to obtain the marginalized PDF in the $\Omega_m - w_x$ plane. Adding in quadrature, the systematic errors identified above to the positional uncertainties, results in a best model for Abell 1689 with a well-defined though broad degeneracy between Ω_m and w_x . The “concordance” model of $\Omega_m \sim 0.3$ and $w_x \sim -1$ lies within the 1σ contour, but the way the degeneracy is pushed against the prior limits suggests either a bias in the mass modeling or misidentified images (fig. S5). Our simulations show that the errors in photometric redshift determination methods at present are too large and limit the efficiency of our technique and introduce biases (see SOM). Therefore, we excluded all images with photometric redshifts in our modeling, bringing down the number of image families used from 34 to 24. Even with this cut, some images were badly reproduced with very high root-mean-square positional deviations—in particular, those that lie in complex crowded regions of the cluster (regions where the signal-

to-noise ratio of the mass map is low due to the presence of several bright cluster galaxies in close proximity). These outlier images from 16 families highlight complex regions in the lens mass distribution, not handled adequately by our simple parametric model. The outlier image systems also tend to have higher redshifts; thus, they are likely to be more affected by uncertainties in modeling the intervening line-of-sight structure as well. We thus deliberately disregarded images that lie in the most complex regions in the mass distribution. Although the recovery of cosmological parameters is insensitive to the choice of profile, both the PIEMD (Pseudo-Isothermal Elliptical Mass Distribution) and NFW (Navarro-Frenk-White) provide comparable constraints on w_x and Ω_m (fig. S3); observationally, some clusters are better fit to one or the other model. Abell 1689 is best fit with a PIEMD profile. The final culled image catalog thus contains 28 images, derived from 12 families at redshifts ranging from $z_S = 1.15$ to $z_S = 4.86$, all of which are spectroscopically measured (table S1 and Fig. 1).

Optimizing our model with all the spectroscopically selected images, including outlier images, does not result in significant constraints on either Ω_m or w_x and yields an averaged reduced $\chi^2 = 0.08$. This indicates that the line of sight and scaling relation errors are likely overestimated. Thus, we optimized again but this time excluded the outlier images. In this iteration, we obtained an averaged reduced $\chi^2 \sim 28$, indicating that now the errors were somewhat underestimated (Fig. 2). Owing to the large estimates for the modeling errors, constraints obtained in this fashion from a single cluster lens are fairly modest. However, combining our results with those from x-ray clusters and WMAP5 leads to $\Omega_m = 0.25 \pm 0.05$ and $w_x = -0.97 \pm 0.07$ (Fig. 2), which is consistent with the values derived from combining WMAP5 with supernovae and BAO, $\Omega_m = 0.265 \pm 0.16 \pm 0.025$ and $w_x = -0.96 \pm 0.06 \pm 0.12$ (39).

Our results—when combined with the results from WMAP5 (40), the supernovae “Gold sample” (4), the Supernovae Legacy Survey (SNLS) (41), the ESSENCE Supernova Survey (SNEssence) (42), and the BAO peak from the Sloan Digital Sky Survey (SDSS) (16)—give $0.23 < \Omega_m < 0.33$ and $-1.12 < w_x < -0.82$ at the 99% confidence level (Fig. 3). This combination of all current viable probes brings down the overall error in w_x by about 30%. Therefore, the combination of cluster methods with WMAP5 has comparable potency to the combination of other cosmological probes.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5994/924/DC1
Methods

Figs. S1 to S5
Tables S1 and S2
References

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Dual Jets from Binary Black Holes

Carlos Palenzuela,^{1,2} Luis Lehner,^{3,4,5*} Steven L. Liebling⁶

The coalescence of supermassive black holes—a natural outcome when galaxies merge—should produce gravitational waves and would likely be associated with energetic electromagnetic events. We have studied the coalescence of such binary black holes within an external magnetic field produced by the expected circumbinary disk surrounding them. Solving the Einstein equations to describe black holes interacting with surrounding plasma, we present numerical evidence for possible jets driven by these systems. Extending the process described by Blandford and Znajek for a single, spinning black hole, the picture that emerges suggests that the electromagnetic field extracts energy from the orbiting black holes, which ultimately merge and settle into the standard Blandford-Znajek scenario. Emissions along these jets could potentially be observable at large distances.

Among the most spectacular of astrophysical events are observations of tremendous amounts of energy careening out along highly collimated jets from a localized central region. Within this central region is the engine behind the jets, generally believed to be a spinning black hole, which helps convert binding and rotational energy into kinetic and thermal energy of the surrounding plasma [e.g., (1)]. This basic picture is widely accepted, but a detailed understanding of these systems remains elusive, in part because of the inability to detect clean electromagnetic signals from the depths of the central engine. Such understanding is particularly needed for phenomena such as gamma-ray bursts and active galactic nuclei, and will likely rely on a combination of sophisticated theoretical analysis along with detailed observations.

Here, we examined the interaction of a binary black hole system with an environment filled with a conducting plasma and a magnetic field as the black holes orbit around each other, ultimately colliding and merging. Such a system is expected as a natural consequence of galaxy mergers. Through a variety of viscous processes, the orbit of the black holes tightens until its dynamics is governed by the time scale of gravitational radiation, disconnected from the properties of the disk (2). The emission of gravitational waves carries off both energy and angular momentum from the system, driving the binary to merger.

The scenario of orbiting binary black holes occurring in vacuum is now well understood thanks to concentrated efforts on both analytical and numerical fronts [see, e.g., (3) and references

therein]. Assuming the binary is immersed in vacuum is generally an excellent approximation because the gravitational wave signal is dominated by the black holes, the inertia of which dominates that of anything else in the problem. A next step, however, is to study possible electromagnetic counterparts that would allow for studying key systems via both bands. Such a possibility is quite likely in the context of supermassive binary black hole mergers, because merging black holes will interact with, at the very least, (i) a circumbinary accretion disk (2), (ii) remnant gas between the black holes (4, 5), and (iii) a magnetosphere (6). All these interactions could potentially contribute to electromagnetic emissions [e.g., (7–19)]. As discussed in (2), as the binary tightens, a common circumbinary disk will be formed within which the black holes will eventually merge. Such a disk will typically be magnetized, anchoring a magnetic field that will permeate the evacuated

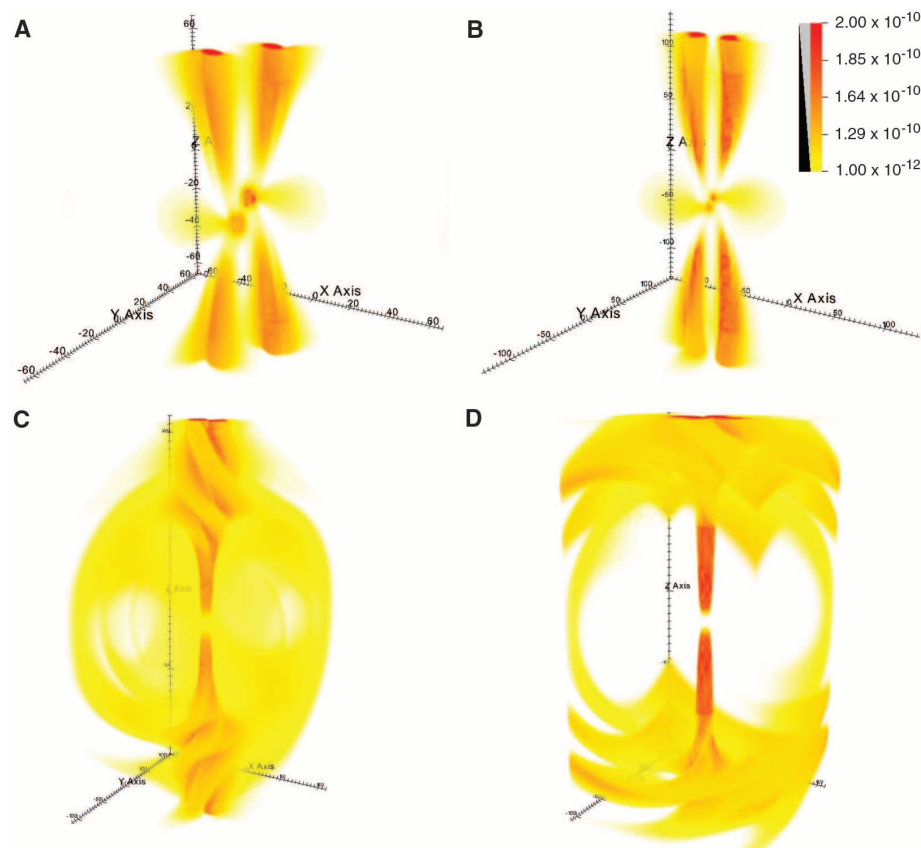


Fig. 1. The radial electromagnetic energy flux F_{EM} at different times. The collimated part is formed by two tubes orbiting around each other following the motion of the black holes. A strong isotropic emission occurred at the time of merger, followed by a single collimated tube as described by the Blandford-Znajek scenario. (A) $t = -11.0(M/10^8 M_\odot)$ hours; (B) $t = -3.0(M/10^8 M_\odot)$ hours; (C) $t = 4.6(M/10^8 M_\odot)$ hours; and (D) $t = 6.8(M/10^8 M_\odot)$ hours.

¹Canadian Institute for Theoretical Astrophysics, Toronto, Ontario M5S 3H8, Canada. ²Department of Physics and Astronomy, Louisiana State University, Baton Rouge, LA 70803, USA. ³Perimeter Institute for Theoretical Physics, Waterloo, Ontario N2L 2Y5, Canada. ⁴Department of Physics, University of Guelph, Guelph, Ontario N1G 2W1, Canada. ⁵Cosmology and Gravity Program, Canadian Institute for Advanced Research, Canada. ⁶Department of Physics, Long Island University, Brookville, NY 11548, USA.

*To whom correspondence should be addressed. E-mail: llehner@perimeterinstitute.ca

region inside the disk containing the black holes and may lie quite far from the orbiting system. Once the black holes merge, the generic outcome will be a spinning black hole within the magnetic field anchored by the disk.

It is precisely this system that was studied in the seminal work of Blandford and Znajek (6) and a large number of subsequent works. In this model, the vacuum outside a spinning black hole immersed in an external magnetic field becomes unstable as the result of a process outlined in (6). Any stray charged particles in the vicinity of the black hole will be accelerated, and this acceleration results in radiation energetic enough to produce electron-positron pairs. This process then repeats, resulting in a cascading effect that populates the region with a conducting plasma (1). As a result of the negligible fluid inertia, the bulk of such plasma experiences essentially no Lorentz force (20), and so the system can be cleanly studied with the so-called force-free approximation (6, 21). This approximation, together with the assumption of stationarity and axisymmetry, allows one to determine that a net outward electromagnetic flux is produced that extracts rotational energy from the black hole. Furthermore, the electromagnetic energy flux is highly collimated and eventually will be transferred into kinetic energy of the plasma, accelerating charges and producing synchrotron radiation. Support for this basic picture has been provided in recent years through numerical models (22–26).

The Blandford-Znajek model is not directly applicable to the very dynamical merger of two black holes. Instead, we used numerical simulations to explore the interaction of a surrounding plasma and magnetic field with a merging black hole binary. We solved the Einstein-Maxwell system of equations coupled to a low-density plasma with negligible inertia in comparison to electromagnetic stresses (20).

We assumed the existence of a circumbinary disk far outside our binary system (and outside our computational domain). The current in this disk produced and anchored a dipolar magnetic field permeating the domain of our computation. This magnetic field was incorporated as both an initial condition and a boundary condition with strength given by a constant B_0 . We adopted black holes in a quasi-circular orbit (which is a reasonable approximation as gravitational wave emission circularizes the orbit). We studied the late orbiting stage and merger, and so we considered an initial separation that gave rise to more than one orbit before the merger took place. Furthermore, we adopted equal-mass [$M_{\text{BH}} = M/2$, with a radius $R_{\text{BH}} = 10(M/10^8 M_\odot)$ AU, where $M_\odot$ is the mass of the Sun], nonspinning black holes to disentangle orbital effects from those that would be induced by any individual spins (i.e., individual Blandford-Znajek effects that would be present around each spinning black hole).

The two black holes orbited and merged within roughly one orbital period [$\sim 13.6(M/10^8 M_\odot)$ hours], after which the final black hole radiated

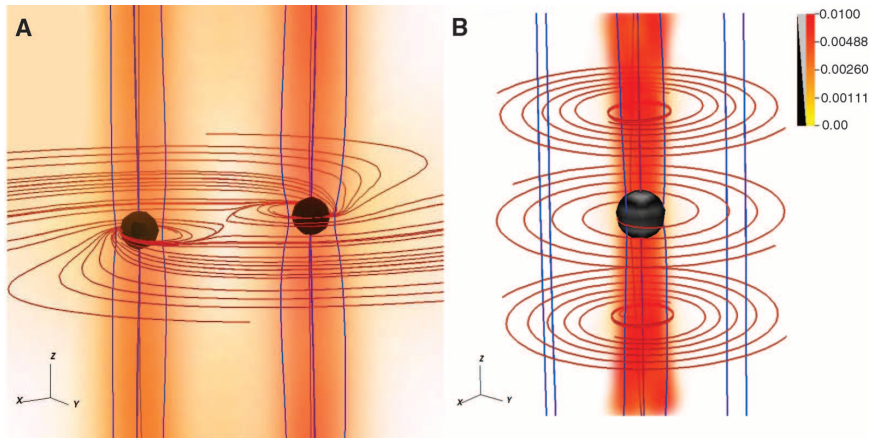


Fig. 2. Snapshots of the electric and magnetic field lines before and after merger. Representative electric field lines are shown in red and a few magnetic field lines are shown in blue. The electromagnetic rotation frequency, Ω_F , is shaded according to the color map. (A) $t = -8.2(M/10^8 M_\odot)$ hours; (B) $t = 4.6(M/10^8 M_\odot)$ hours.

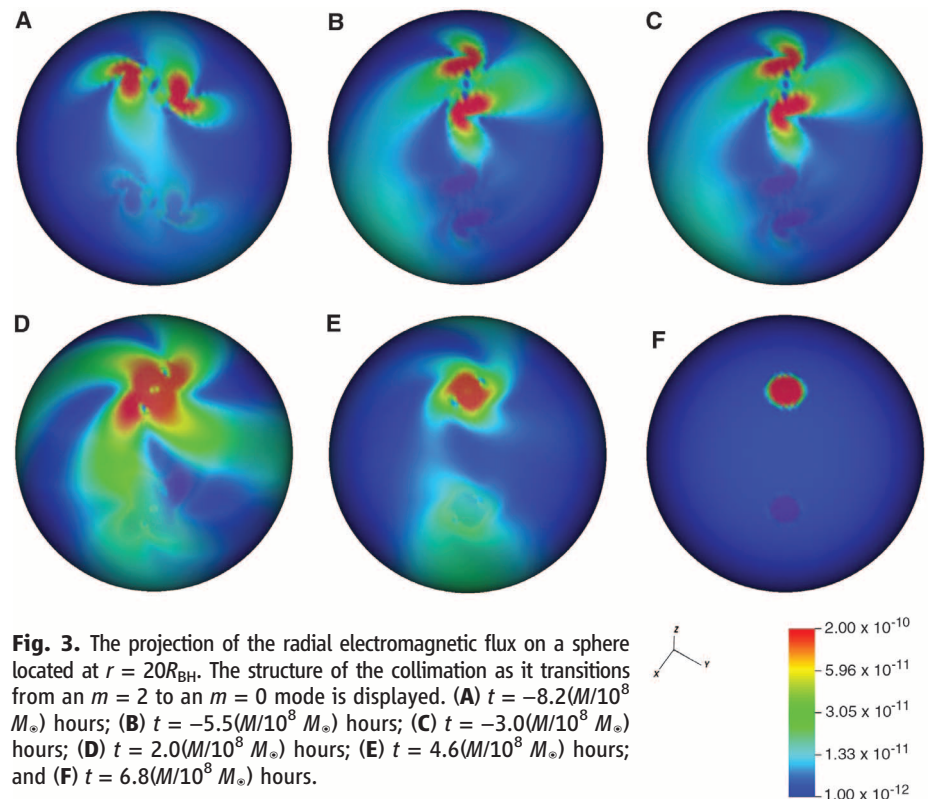


Fig. 3. The projection of the radial electromagnetic flux on a sphere located at $r = 20R_{\text{BH}}$. The structure of the collimation as it transitions from an $m = 2$ to an $m = 0$ mode is displayed. (A) $t = -8.2(M/10^8 M_\odot)$ hours; (B) $t = -5.5(M/10^8 M_\odot)$ hours; (C) $t = -3.0(M/10^8 M_\odot)$ hours; (D) $t = 2.0(M/10^8 M_\odot)$ hours; (E) $t = 4.6(M/10^8 M_\odot)$ hours; and (F) $t = 6.8(M/10^8 M_\odot)$ hours.

its excess structure, settling into a Kerr black hole with spin $a \equiv Jc/(GM^2) \approx 0.67$, where J is the black hole's angular momentum, G is Newton's constant, and c is the speed of light (Fig. 1, fig. S1, and movies S1 and S2). To study the system in more detail, we monitored the Newman-Penrose scalars Φ_2 and Ψ_4 (the square of the former and the integral of the latter describe the electromagnetic and gravitational energy fluxes, respectively); the Poynting flux; the function $\Omega_F \equiv F_{\theta\phi}/F_{\theta\theta}$ [which in axisymmetric, stationary scenarios describes the angular velocity of magnetic

field lines (6)]; and the electric and magnetic field topologies.

In the first stage, before merging, the black holes stirred the surrounding plasma (Fig. 2). The orbital dynamics and strong curvature affected the electromagnetic field in the close neighborhood of each black hole, inducing both a poloidal electric field and a toroidal magnetic field—both scaling as vB_0 [as in models for magnetospheric interactions of binary pulsars (27, 28)]. The induced time-dependent topology gave rise to a net Poynting flux aligned/antialigned with the orbital

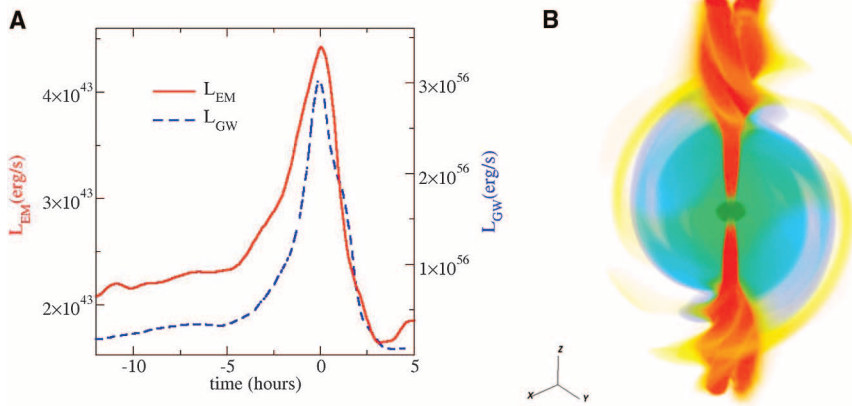


Fig. 4. (A) The gravitational and the (collimated) electromagnetic luminosities for the case $B_0 = 10^4$ G and $M = 10^8 M_\odot$. (B) Electromagnetic (yellow-red) and gravitational (green-blue) radiation from the system just after the merger at $t = 4.6(M/10^8 M_\odot)$ hours.

momentum vector around each black hole, with an electromagnetic frequency given by $\Omega_F \approx \Omega_{\text{orb}}/5$. Thus, even though the black holes were not spinning, there was a strongly collimated electromagnetic flux of energy dominated by an $m = 2$ multipolar structure with a time dependence determined by the orbital motion.

During the merger, which lasted $\sim 7(M/10^8 M_\odot)$ hours—from late orbiting through the plunge phase to the formation of a highly distorted black hole—the highly nonlinear dynamics translated into a substantial increase in both electromagnetic and gravitational energy radiated. As expected, a common, collimated tube arose as the flux transitioned from $m = 2$ to $m = 0$. A roughly isotropic flux was also emitted. During the inspiral, the isotropic component of the flux was much smaller than that of the collimated component. During the merger, however, both of these energy fluxes reached a peak and the collimated part doubled in magnitude.

The final stage was described by a single black hole, which, after a relatively short time, was well approximated by a Kerr black hole. As expected, the remnant black hole radiated gravitational radiation settling into a standard Kerr configuration. Thus, the post-merger behavior of the electromagnetic field behavior was increasingly better represented by the Blandford-Znajek process for a spinning black hole with $a \approx 0.67$. As a result, the collimated electromagnetic energy flux did not decay to zero but rather approached the value predicted by the Blandford-Znajek model. For a single spinning black hole, this energy flux evaluated at the horizon scales as $F_{\text{EM}} \approx R_{\text{BH}} \Omega_F (\Omega_H - \Omega_F) B^2$, where Ω_H is the rotation frequency of the black hole (which is similar to the orbital velocity at the merger). In accordance with this model, the electromagnetic rotation frequency for the final black hole relaxed to $\Omega_F \approx \Omega_H/2$.

Energetically, the system radiated gravitational waves primarily through $l = |m| = 2$ modes. These waves displayed a chirping behavior as the orbit tightened, followed by exponential decay after the merger [e.g., (3)]. Overall, the system radiated

$\sim 2.5\%$ and $\sim 16\%$ of the rest mass energy and angular momentum (at the initial separation), respectively. In the electromagnetic band, the radiation profile displayed a more complex structure.

Projected over a sphere located at $r = 20R_{\text{BH}}$, the electromagnetic energy flux shows clear collimation, evident as bright spots (Fig. 3). These spots are symmetric with respect to the orbital plane and revolve around each other as a result of the orbiting until they merge along the poles. Thus, the behavior of the electromagnetic fields was tightly tied to the dynamics of the binary, making them excellent tracers of the spacetime. Indeed, the resulting tubes of collimated flux resemble the familiar “pair of pants” picture of the event horizon for the merger of a binary black hole system in vacuum (29).

The total energy flux in both the electromagnetic and gravitational wavebands for a particular astrophysically relevant case with $M = 10^8 M_\odot$ and $B = 10^4$ G [i.e., not exceeding the Eddington magnetic field strength $B \approx 6 \times 10^4 (M/10^8 M_\odot)^{-1/2}$ G (30)] showed a clear sweep upward, apparent in both channels at the time of merger. Afterward, both decreased rapidly. However, while the gravitational flux essentially shuts off shortly after merger (as the system relaxes to a stationary black hole), the electromagnetic flux rises up to the level predicted by the Blandford-Znajek mechanism (Fig. 4).

Overall, the total radiated electromagnetic energy in the late stages behaved according to

$$\frac{E_{\text{rad}}^{\text{em}}}{Mc^2} = 10^{-14} \left(\frac{M}{10^8 M_\odot} \right)^2 \left(\frac{B}{10^4 \text{ G}} \right)^2 \quad (1)$$

This is about an order of magnitude larger than the same system studied in the electrovacuum case (i.e., not including the plasma) (17, 18), indicating that the plasma taps the orbital/rotational energy from the system more efficiently. This late-stage radiation, which is mostly isotropic, can potentially couple to the circumbinary disk via magnetosonic waves that propagate perpendicular to the magnetic field lines. If the viscous time scales of the disk become similar to, or

larger than, the magnetic transport time scale [see section VI of (31) for definitions of these time scales], then these waves could affect the dynamics of the disk, and this coupling could potentially manifest in a variability in the disk’s emissions, indicating that the black holes are merging.

During the last hours before the merger, the jets have a luminosity of 2×10^{43} to 4×10^{43} erg/s ≈ 0.0015 to $0.0030 L_{\text{Edd}}$ (where L_{Edd} is the Eddington luminosity). This electromagnetic energy can be transferred to kinetic energy of the plasma, which will radiate through synchrotron processes in frequencies around the gigahertz region. Such a flux of energy implies that it would be possible to observe these systems to $z \approx 1$ with future x-ray telescopes. The system will be sufficiently bright for hours before the merger and remain so through the merger (Fig. 4). This radiated power has a time dependence given by $(R_{\text{orb}} \Omega_{\text{orb}})^2 B^2$, and the flux exhibits a clear transition $m = 2 \rightarrow 0$ at the time of the merger.

Furthermore, joint detections in both electric and gravitational wave bands (the power of the latter scales as $R_{\text{orb}}^4 \Omega_{\text{orb}}^6 M^2$) are therefore quite probable, as future space-based interferometers will be capable of observing supermassive binary black hole systems for weeks, perhaps months, before merger and for a considerable time afterward. Gravitational waves from supermassive binary systems could be detected as far as redshifts of $z \approx 5$ to 10 (32), although good sky localization will require electromagnetic counterparts. Electromagnetic luminosities of $\sim 10^{43}$ erg/s correspond to an isotropic bolometric flux of $F_x \approx 10^{-15}$ erg cm $^{-2}$ s $^{-1}$ that could be detected to redshifts of $z \approx 1$ and even further, depending on anisotropies.

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Supporting Online Material

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Materials and Methods

Fig. S1

Movies S1 and S2

References

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Unidirectional Emission of a Quantum Dot Coupled to a Nanoantenna

Alberto G. Curto,¹ Giorgio Volpe,¹ Tim H. Taminiau,¹ Mark P. Kreuzer,¹ Romain Quidant,^{1,2} Niek F. van Hulst^{1,2*}

Nanoscale quantum emitters are key elements in quantum optics and sensing. However, efficient optical excitation and detection of such emitters involves large solid angles because their interaction with freely propagating light is omnidirectional. Here, we present unidirectional emission of a single emitter by coupling to a nanofabricated Yagi-Uda antenna. A quantum dot is placed in the near field of the antenna so that it drives the resonant feed element of the antenna. The resulting quantum-dot luminescence is strongly polarized and highly directed into a narrow forward angular cone. The directionality of the quantum dot can be controlled by tuning the antenna dimensions. Our results show the potential of optical antennas to communicate energy to, from, and between nano-emitters.

Optical antennas, acting as converters between propagating and localized fields, provide an effective route to couple photons in and out of nanoscale objects. These antennas are the counterparts of conventional radio and microwave antennas and operate in the visible regime (1, 2). Optical antennas have been shown to focus optical fields to subdiffraction-limited volumes (3), enhance the excitation and emission of quantum emitters (4–7), and modify their spectra (8).

A characteristic of antennas is their directed emission and reception. So far, the control of directionality has mainly been pursued by photonic crystal structures (9) and surface-plasmon-based devices (10–12). However, for such structures approaching the nanometer scale diffraction can limit the collimated beaming of light. On the other hand, the interaction of quantum emitters with light is best enhanced with microcavities (13, 14). Compared with these approaches, plasmonic nanoantennas offer a much smaller footprint in an open geometry combining strong subwavelength fields and increased transition rates, together with the prospect of directionality.

Earlier work (15–17) observed variations in the angular emission of molecules by the presence of metallic objects in their near field. From experience at radio and microwave frequencies, it

is known that highly directed beams are commonly obtained with Yagi-Uda antennas (18). In the optical regime, directional far-field scattering of a polarized laser beam from an array of Yagi-Uda antennas has been recently presented (19). Complete control of the direction of light emission from a single quantum emitter using an optical Yagi-Uda antenna has been theoretically proposed (20–22).

We report the realization and observation of unidirectional nanoscale photon sources by coupling single-quantum systems to a Yagi-Uda design. The realization of such a source requires the precise near-field coupling of an emitter to a nanoscale antenna that is tuned to the emission spectrum. To this end, a quantum dot (QD) is placed at the resonant feed element of a nano-fabricated Yagi-Uda antenna.

A Yagi-Uda antenna consists of an actively driven feed element surrounded by a set of parasitic elements acting as reflectors and directors (Fig. 1A). The reflectors and directors are de-

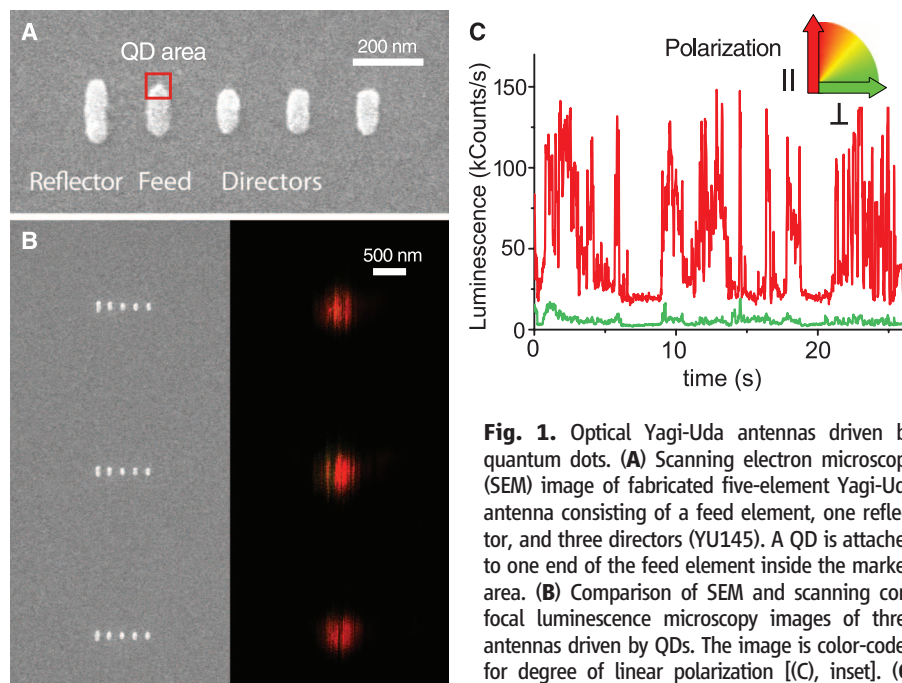


Fig. 1. Optical Yagi-Uda antennas driven by quantum dots. (A) Scanning electron microscopy (SEM) image of fabricated five-element Yagi-Uda antenna consisting of a feed element, one reflector, and three directors (YU145). A QD is attached to one end of the feed element inside the marked area. (B) Comparison of SEM and scanning confocal luminescence microscopy images of three antennas driven by QDs. The image is color-coded for degree of linear polarization [(C), inset]. (C) Intensity time trace of luminescence in both polarization channels for one of the antennas in (B), showing blinking of a single QD.

¹Institut de Ciències Fotoniques (ICFO), Mediterranean Technology Park, 08860 Castelldefels (Barcelona), Spain. ²Institució Catalana de Recerca i Estudis Avançats (ICREA), 08015 Barcelona, Spain.

*To whom correspondence should be addressed. E-mail: niek.vanhulst@icfo.es

tuned in length with respect to the dipolar resonance of the feed, and the spacing between elements is chosen so that a traveling wave pointing toward the directors is created. Numerical simulations were used to design five-element gold Yagi-Uda antennas for operation wavelengths of ~ 800 nm (23).

To obtain a strong near-field coupling of a QD to the antenna mode, it is critical to place the emitter at a position of high electric mode density—that is, at one of the ends of the feed element. We used a two-step electron beam lithography process combined with chemical functionalization (23). The first lithography step defined the antenna structures on a glass substrate, followed by thermal evaporation of a 30-nm layer of gold. The second lithography step set the boundaries for the formation of a self-assembled monolayer of mercaptoundecanoic acid exclusively on the predefined exposed areas. Core-shell QDs were immobilized on the functionalized areas (70-nm squares), and the remaining resist was removed. A typical fabricated antenna is shown (Fig. 1A) with feed length $L_f = 145$ nm, spacing $a_r = 175$ nm between reflector and feed, and spacing $a_d = 200$ nm between directors. The total length is 830 nm, which is approximately one emission wavelength.

The emission of QDs on antennas was characterized with a confocal microscope with three dedicated detection branches: for luminescence imaging, angular detection, and spectroscopy, respectively (23). The use of a high-numerical aperture (NA) objective (1.46 NA) is essential for the angular detection. The sample is excited by a circularly polarized He-Ne laser beam [wavelength (λ) = 633 nm], which is focused to address a single antenna at a time. The resulting luminescence is separated from the excitation wavelength with a dichroic mirror and a long-pass filter. For confocal detection, the use of a polarizing beam-splitter and two detection channels ($I_{\parallel}$ and $I_{\perp}$) with avalanche photodiodes allows us to determine polarization anisotropies. For detection of the angular emission, we recorded images of the intensity distribution on the back focal plane of the high-NA objective (conoscopy) (24) on an electron-multiplying charge-coupled device (emCCD) camera. These Fourier-plane images (momentum space) contain the directions of emission toward the substrate.

All presented confocal luminescence images are color-coded (red-yellow-green) for the degree of linear polarization (DOLP) [$\text{DOLP} = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp})$], with red being linear polarization parallel to the feed longitudinal axis. All antenna-QD

systems in Fig. 1B show red (linearly polarized), confirming that the QD drives the linear dipole of the feed element (15, 25). The QDs are cleanly positioned on the antennas, and the emission arises from quantum emitters as discrete blinking events interrupt the signal during raster-scanning of the sample. This blinking is better seen in the luminescence time trace displayed in Fig. 1C, in which clear on and off states can be identified, which is characteristic of a single emitter. From many such time traces, we conclude that single QDs occur frequently, whereas the typical number of QDs observed is one, two, or three—a number that can be controlled by both the size of the functionalized area and the concentration of the deposited QD solution.

To gain direct insight into the changes in the QD emission upon coupling to the antenna, we compared three types of nanostructures (Fig. 2, A and B): small 60-nm gold squares, $\lambda/2$ dipole antennas, and the Yagi-Uda antennas. The small, off-resonant squares were taken as a reference for QDs on metal; the dipole antennas are essentially the feed element of a Yagi-Uda antenna. In the reference case, the polarization of the luminescence varies, with a DOLP ranging from -0.5 to 0.5 (Fig. 2A, first column) because the QDs have different orientations and the gold squares induce no preferential direction. The corresponding emission pattern is shown in Fig. 2B. The momentum space images contain two distinct circles in the polar angle θ : The outer circle is the maximum collection angle of our objective ($\theta_{\text{NA}} = 72.8^\circ$), whereas the inner circle is the critical angle for the glass-air interface ($\theta_c = 41.1^\circ$). A dipole close to an interface emits primarily into the high-index medium, with sharply peaked maxima at the critical angle. Moreover, our QDs exhibit a degenerate transition dipole moment contained on a “bright” plane (26, 27). As a result, the radiation pattern of a QD is nearly isotropic in the azimuthal angle ϕ .

When coupled to a $\lambda/2$ dipole resonant nano-antenna (Fig. 2, second column), the picture changes dramatically: The QD luminescence turns into a clear linear polarization parallel to the long axis of the antenna ($\text{DOLP} \approx 0.8$), and the radiation pattern transforms to that of a linear dipole close to an interface (24). These are two clear signatures of the near-field coupling (15). The QD emission becomes fully determined by the antenna mode, both in polarization and direction, despite the degeneracy of the QD dipole moment.

We can also control the emission direction by positioning the QDs at one end of the $\lambda/2$ feed element of Yagi-Uda antennas (Fig. 2, third column). The luminescence remains strongly linearly polarized ($\text{DOLP} \approx 0.8$), but the radiation pattern now shows a single lobe, demonstrating the unidirectional emission of a QD due to coupling to an optical antenna.

The directional performance can be quantified by a front-to-back ratio (F/B), defined as the intensity ratio between the point with maximum

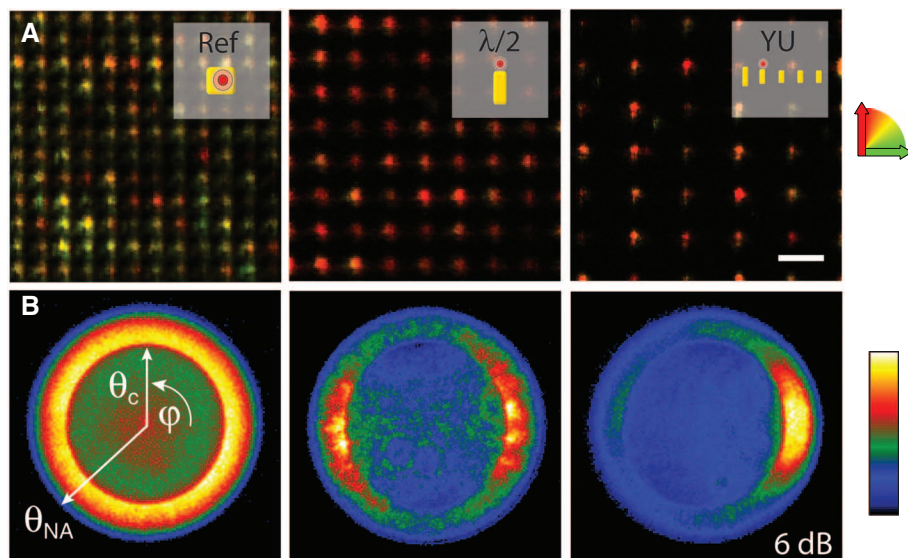
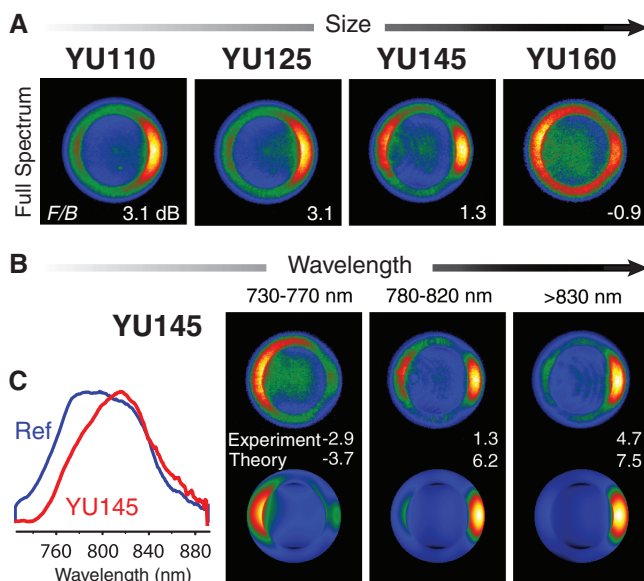


Fig. 2. Unidirectional emission of a QD coupled to an optical Yagi-Uda antenna and comparison with other metal nanostructures. **(A)** Scanning confocal luminescence images of QDs on reference 60-nm gold squares (Ref), half-wave dipole antennas ($\lambda/2$), and Yagi-Uda antennas (YU), respectively. Different colors show variations in degree of linear polarization. YU corresponds to antennas, with the parameter set YU145. The schematics in the insets are not to scale. Scale bar, 2 μm . **(B)** Radiation pattern (intensity distribution at the back focal plane of the objective) from an individual structure in (A). Critical (θ_c) and numerical aperture (θ_{NA}) angles are indicated. For YU, a 830-nm long-pass filter was used. **(C)** Angular radiation pattern in the polar angle (θ) for the Yagi-Uda antenna (black), which is in good agreement with the theoretical prediction (red).

Fig. 3. Tuning of optical Yagi-Uda antennas. **(A)** Radiation patterns in the back focal plane of the objective for four different individual Yagi-Uda antennas of increasing overall size, characterized by the length of the feed element L_f from 110 to 160 nm. The full emission spectrum was recorded. **(B)** Angular radiation patterns for different spectral detection windows for the YU145 shown in (A). Theory and experiment are compared. The front-to-back ratio in decibels is indicated for each pattern. **(C)** Luminescence spectra of QDs coupled to the reference and YU145.



emitted power and the point diametrically opposite in the radiation pattern. The F/B value is essentially 0 dB for the reference squares and the dipole antennas, as expected for symmetrical structures. For the Yagi-Uda antenna in Fig. 2B, F/B is 6.0 dB. The directed emission is centered at $\theta = 49.4^\circ$, with a beam half width at half maximum of 12.5° in θ and 37.0° in ϕ . The experimental angular radiation pattern can be calculated from the Fourier-plane image (23) and agrees well with the theoretical prediction (Fig. 2D). The simulations quantify that as much as 83.2% of the QD emission is directed into the high-index glass substrate. The emission is thus truly unidirectional, unlike configurations with a low-index contrast interface, which results in two separate emission lobes (19). The total brightness of the QDs coupled to Yagi-Uda antennas is comparable with that of dipole antennas. This observation is in agreement with our calculations and previous theoretical predictions (21), which show that adding the parasitic antenna elements does not strongly reduce the antenna radiation efficiency (only a 10 to 20% reduction). The Yagi-Uda antenna redirects the emission instead of suppressing one half of the radiation. Because of excitation enhancement, the number of counts detected from both types of antennas is much higher than for a QD on glass without coupling to an antenna.

Any Yagi-Uda antenna is designed to operate at a certain frequency and bandwidth. To assess the frequency dependence of the directionality, we fabricated Yagi-Uda antennas tuned to four different resonant frequencies (Fig. 3A), identified with their feed element lengths L_f : 110 to 160 nm (a complete parameter can be found in table S1). Increasing the antenna length creates a redshift of the resonance. The shortest antennas (YU110 and YU125) emit clearly unidirectionally. These antennas are tuned close to the QD emission, and their bandwidth contains the

complete luminescence spectrum. These observations are in agreement with a calculated bandwidth of 150 nm for end-fire operation, defined as $F/B > 3$ dB. On the other hand, for longer antennas (YU145 and YU160) the antenna resonance is more detuned from the QD emission, and directivity is hindered by short-wavelength components of the spectrum. This spectral dependence does also occur for radio frequency Yagi-Uda antennas (28) and was in fact predicted for the optical regime (20, 29).

To corroborate the analysis above, we evaluated the dependence of the directionality on the wavelength of the emitted photons for a given antenna, YU145 (Fig. 3, B and C). This antenna was red-detuned from the QD emission. As a result, the QD emission spectrum was modified by the near-field coupling to the antenna (Fig. 3C) (8). The emission spectrum of an individual YU145 antenna was divided in three parts by using three different filters. Although hardly any directed emission was observed for the complete spectrum (Fig. 3A), a high directivity was recovered when only the long-wavelength part of the spectrum was selected (Fig. 3B). For short wavelengths—below a cut-off value—the emission was even reversed, with the main lobe pointing backward. Similar behavior was observed for the longer antenna YU160, with all characteristic wavelengths shifted to longer wavelengths. The numerically calculated back-focal-plane images agree well with the experimental results (Fig. 3B). The maximum experimental F/B values tend to be lower than the theoretical values because part of the signal stems from autoluminescence of the gold nanoparticles and a fraction of the QD emission is not coupled to the antenna, both of which contribute to a minor isotropic background. Thus, by tuning the antenna or selecting certain emission bands the frequency dependence of the directivity of an optical Yagi-Uda antenna is proved.

The presented antenna transforms the non-directional QD luminescence into a directed light source that can be efficiently collected, simply with a low NA. Further optimization of the many Yagi-Uda design parameters, including the addition of more director elements, might sharpen up the unidirectional cone and tune the central emission angle. The operation bandwidth can be increased by the use of a log-periodic design (2). By reciprocity, the antennas should work both in emission and absorption. The near-field coupling to the antenna plasmon resonance enhances radiative transition rates, increasing the emission efficiency. All this control over photon emission is obtained from an antenna that is only a single wavelength long. Unidirectional optical antennas thus provide a route to effectively communicate light to, from, and between nano-emitters, for example in directed, bright single-photon sources for quantum optical technologies, planar biochemical sensors, and light-harvesting and emission devices.

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Ceria Maintains Smaller Metal Catalyst Particles by Strong Metal-Support Bonding

Jason A. Farmer and Charles T. Campbell*

The energies of silver (Ag) atoms in Ag nanoparticles supported on different cerium and magnesium oxide surfaces, determined from previous calorimetric measurements of metal adsorption energies, were analyzed with respect to particle size. Their stability was found to increase with particle size below 5000 atoms per particle. Silver nanoparticles of any given size below 1000 atoms had much higher stability (30 to 70 kilojoules per mole of silver atoms) on reduced $\text{CeO}_2(111)$ than on $\text{MgO}(100)$. This effect is the result of the very large adhesion energy (~ 2.3 joules per square meter) of Ag nanoparticles to reduced $\text{CeO}_2(111)$, which we found to be a result of strong bonding to both defects and $\text{CeO}_2(111)$ terraces, apparently localized by lattice strain. These results explain the unusual sinter resistance of late transition metal catalysts when supported on ceria.

Nanoparticles of late transition metals adsorbed on oxide surfaces form the basis for many catalysts important in energy technology, pollution prevention, and environmental cleanup. The catalytic activity per surface metal atom and selectivity can depend strongly on the particle size below 6 nm (1–5), the choice of oxide support (1, 2, 6–21), and the extent of oxide reduction (22–26). Furthermore, the metal nanoparticles often sinter—they form fewer, larger particles—under catalytic reaction conditions and even during catalyst preparation. Sintering results in loss of activity or selectivity, mainly through a decrease in the number of exposed metal atoms but also through the loss of the smallest particles, which may have electronic properties that make them especially reactive. Late transition metal catalysts have also been reported to sinter more slowly or maintain smaller particles when supported on CeO_2 relative to other supports (16, 19, 21). To fundamentally understand such structure-reactivity relations in catalytic phenomena, it is important to know how the energy of transition metal atoms in catalyst nanoparticles depends on the particle size and the nature of the oxide support surface to which they bind.

Here, we analyzed our previous calorimetric measurements of the adsorption energies of Ag vapor onto different oxide surfaces, on which Ag nanoparticles grow with very similar size and

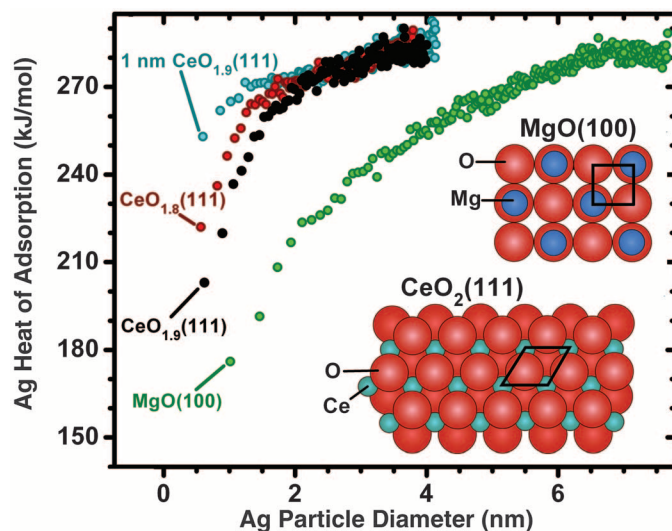
number density upon adsorption. These data allow for a quantitative comparison of variations of the energy of Ag atoms in Ag nanoparticles of different sizes. We next compared how the stability of a metal particle of a given size depends on the nature of the oxide surface to which it is attached. This analysis provides a quantitative estimate of the underlying thermodynamic reasons why late transition metal catalysts maintain smaller particle sizes and better resist sintering when on ceria supports relative to other oxide materials. By comparing ceria surfaces with different extents of reduction, we could assess qualitatively the stabilizing effect of surface oxygen vacancies on the adhesion energy of Ag nanoparticles to ceria. These results help to explain why

ceria often enhances the performance of late transition metal catalysts—especially their unusual sinter resistance relative to other supports—in a wide variety of reactions in energy and environmental technology (8, 11, 13, 15–21). The results also show that metal-oxide adhesion is locally stronger around oxide defects.

Our calorimetric measurements of the adsorption energies of Ag gas atoms onto clean and ordered surfaces of $\text{CeO}_{2-x}(111)$ films (with $x = 0.1$ and 0.2) are described in detail elsewhere (27). The $\text{CeO}_{2-x}(111)$ films were grown on $\text{Pt}(111)$, and results were compared for film thicknesses of 1, 2, 3, and 4 nm (27). The $\text{Ce}(3d)$ region in the X-ray photoelectron spectroscopy (XPS) was used to measure the $\text{Ce}^{3+}/\text{Ce}^{4+}$ concentration ratio within the probe depth of XPS (~ 1 nm), as described in (28), which was found to be 1:4 for the as-grown films. We describe these surfaces as $\text{CeO}_{2-x}(111)$, with $x = 0.1$, or as $\text{CeO}_{1.9}(111)$, which denotes $\text{CeO}_2(111)$ with 5% oxygen vacancies (29). The total density of steps and kinks was estimated from scanning tunneling microscopy (STM) images and low-energy electron diffraction spot widths to be $\sim 6\%$ of the total surface atoms, and most of the vacancies were probably localized at these step or kink defects (27). Measurements of the $\text{Ce}^{3+}/\text{Ce}^{4+}$ ratio were also performed on such 4-nm films after further thermal reduction by heating in ultrahigh vacuum, for which x was shown to increase to 0.2; we refer to the resulting films as $\text{CeO}_{1.8}(111)$ (27).

The measurements of the heat of adsorption for Ag on $\text{MgO}(100)$ films that we analyze here have also been reported previously (30). The $\text{MgO}(100)$ was 4 nm thick and grown on $\text{Mo}(100)$ (30), and later proved to have $\sim 5\%$ step and kink sites as

Fig. 1. Measured heat of Ag atom adsorption versus the Ag particle diameter to which it adds, for Ag adsorption onto four different surfaces: two 4-nm $\text{CeO}_2(111)$ films with different extents of surface reduction ($x = \sim 0.1$ and 0.2 in CeO_{2-x}), grown on $\text{Pt}(111)$; a 1-nm $\text{CeO}_{1.9}(111)$ film grown on $\text{Pt}(111)$; and a 4-nm $\text{MgO}(100)$ film grown on $\text{Mo}(100)$. The inset shows structural models for perfect $\text{CeO}_2(111)$ and $\text{MgO}(100)$, with their unit cells in black lines.



Department of Chemistry, Box 351700, University of Washington, Seattle, WA 98195, USA.

*To whom correspondence should be addressed. E-mail: campbell@chem.washington.edu

the dominant defects (31). For all four surfaces, the heat of adsorption of Ag was measured in ultrahigh vacuum as a detailed function of the amount of adsorbed Ag per unit area (i.e., Ag coverage). We found that the heat started at some low value depending on the surface, and increased to the heat of sublimation of bulk Ag (285 kJ/mol) as Ag coverage increased (27, 30).

Our previous quantitative analysis of spectral intensities in Auger electron spectroscopy and ion scattering spectroscopy for the different elements as a function of Ag coverage allowed us to ascertain the growth morphology of the Ag films on these MgO(100) and CeO_{2-x}(111) surfaces (27, 30). The data were well fitted on all four surfaces by assuming that the Ag grows as three-dimensional (3D) Ag particles with the shape of a hemispherical cap and a fixed number per unit area, N , independent of Ag coverage. The data are not sensitive to the exact shape of the 3D particles but are very sensitive to their aspect ratio (ratio of average thickness to effective diameter) or thickness/area ratio, so that any shape with the same aspect ratio as hemispherical caps would fit the data equally well if using the same N . Our finding that N was independent of coverage (above ~ 0.03 monolayer) is consistent with the usual finding for such systems that form 3D particles—that is, their number density during nucleation quickly reaches a saturation value and thereafter stays nearly constant (32). The value of N was similar for all four surfaces, equal to 2.5×10^{12} particles/cm² for the MgO(100) surface (30) and 4×10^{12} particles/cm² for all three CeO_{2-x}(111) surfaces (27). Dividing the Ag coverage (atoms/cm²) by N (particles/cm²) gives the average number of Ag atoms per particle at any given Ag coverage, which can be combined with the bulk density of Ag (5.9×10^{22} atoms/cm³) to give the average particle volume. This volume then corresponds to an average Ag particle diameter at each coverage. Because diameter varies only as the inverse cube root of N , the factor of ~ 2 maximum error on N (27) makes only a 25% error in diameter, to which the conclusions below are insensitive.

Figure 1 shows the measured heat of adsorption for these four surfaces plotted versus the average Ag particle diameter to which it adds, using the above approach to convert from Ag coverage to Ag particle diameter. The heat of adsorption versus coverage data were taken from (27, 30). The data in this format show large differences between the different surfaces in the stability of metal atoms adding to particles of the same size, and also show how stability depends on particle size for a given surface. For example, Ag atoms bind more strongly by ~ 75 kJ/mol to a 1-nm Ag particle on thermally reduced CeO_{1.8}(111) than to a particle of the same size on MgO(100), whereas they bind more strongly by ~ 90 kJ/mol to a 5-nm particle than to a 1-nm particle when both particles are on MgO(100).

Another way to view these same data is to plot the energy of a metal atom after it adds to a

particle (relative to its energy in bulk Ag) versus the number of metal atoms in the particle (Fig. 2). Silver atoms are 30 to 70 kJ/mol more stable in Ag nanoparticles for any given size (up to ~ 1000 atoms) when those particles are attached to CeO_{2-x}(111) surfaces than when the particles are attached to MgO(100) surfaces. These differences are present for 4-nm films, well beyond the range where oxide film thickness has a measureable effect (i.e., below 2 nm). The difference decreases for larger particles and essentially disappears by ~ 5000 atoms per particle, where the energy of the added metal atom reaches the stability of the bulk metal even on MgO(100). Given that in reality there is a distribution of metal particle sizes for a given metal coverage, which broadens out the curves in Figs. 1 and 2, these differences between substrates at a given average particle size may be even more striking than shown here.

Also shown in Fig. 2 are the only other data of this type ever reported, for Pb particles at a density of 8×10^{11} particles/cm² on this same type of 4-nm MgO(100) film (33). The Pb energy is plotted relative to its energy in bulk Pb(solid). Note that the Pb and Ag data for MgO(100) fall very close to each other and are distinctly different from the Ag data for any of the CeO_{2-x}(111) surfaces. The similarity in these curves for Ag and Pb on MgO(100) confirms our earlier explanation of this Pb curve as being dominated by the effect of particle size on the number of metal-metal bonds per atom (33). This Ag-MgO curve is above the Pb-MgO curve, as expected because Ag-Ag bonds are stronger than Pb-Pb bonds.

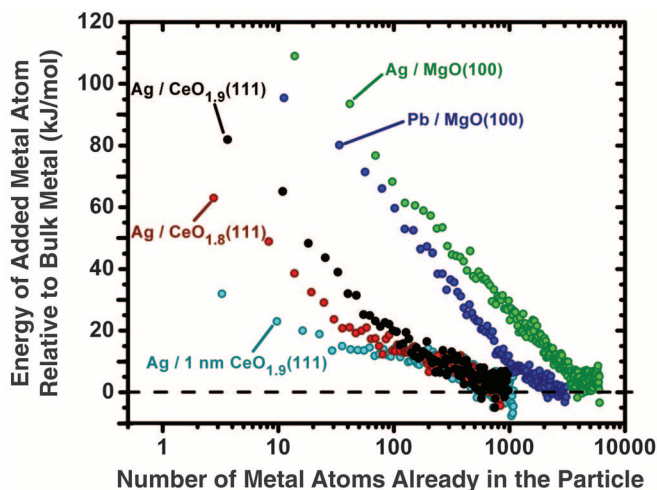
These curves in Fig. 2 directly reflect the thermodynamic driving force for nanoparticle sintering. If a metal atom is less stable on a certain particle size than in the bulk, it prefers to move to a larger particle where it is more stable [i.e., move to lower energy (to the right on each of these curves) until it reaches the minimum, or zero]. This driving force for sintering drops below 10 kJ/mol for ~ 400 -atom (3-nm) particles on all three CeO_{2-x}(111) surfaces, but this requires ~ 3000 -atom (6-nm) particles on MgO(100). Sintering will stop at much

smaller particles on CeO₂ surfaces. Because Au nanoparticles are very active for several catalytic reactions when 3 nm in diameter but almost completely inactive above 6 nm (1–3), this particular size range is very important. We previously derived kinetic rate equations showing that this thermodynamic driving force is exponentially reflected in the sintering rate as if it were a negative activation energy (33, 34), which predicts that the sintering rates for particles below 3 nm will be much slower on CeO₂ surfaces than on MgO(100) (by a factor of at least 30 at 500°C), consistent with observations that ceria offers a more sinter-resistant support for late transition metals than do other oxides (16, 19, 21).

Figures 1 and 2 also show that the stability of Ag atoms in small Ag nanoparticles (<1.5 nm or 30 atoms) on CeO_{2-x}(111) is ~ 15 kJ/mol greater when the oxide surface is thermally reduced to CeO_{1.8}(111). This increase in stability with vacancy concentration is consistent with observations that Au nanoparticles nucleate at oxygen vacancies and vacancy clusters on CeO_{2-x}(111) (35), and with calculations that Ag adatoms bind >100 kJ/mol more strongly to vacancy sites on CeO_{2-x}(111) than to stoichiometric sites (36). Silver atoms in nanoparticles smaller than 1.5 nm (30 atoms) on CeO_{2-x}(111) films are 20 to 50 kJ/mol more stable when the film is only 1 nm thick (versus 4 nm). This difference may be caused by long-range electronic interactions with the underlying Pt(111) substrate. Other related effects have been observed experimentally and theoretically in this oxide thickness range, with the extent of charging of the adsorbed metal even being affected by thickness (37–40). Although we did not test the effect of MgO(100) film thickness on Ag adsorption, we found that the heat of adsorption of Li was higher by ~ 50 kJ/mol on 1-nm MgO(100) films than on 4-nm films (39).

The effect of the different support materials on the energy diagram for the sintering of Ag nanoparticle catalysts is summarized in Fig. 3. The reaction is much more exothermic on MgO(100) (~ 45 kJ/mol) than on the different reduced CeO₂(111)

Fig. 2. Measured energy of a Ag atom, relative to its energy in bulk Ag(solid), versus the Ag particle size to which it adds, for Ag particles on the same four surfaces as in Fig. 1. For comparison, previously published results for Pb adsorption on this same type of MgO(100) film are also shown (33, 41).



surfaces, where a 400-atom Ag particle has very little energy release to drive the sintering reaction [<10 kJ/mol on the $\text{CeO}_{2-x}(111)$ surfaces studied here]. This effect increases with the extent of reduction of the CeO_2 surface, and also increases when the CeO_2 film is so thin (1 nm) that the underlying Pt(111) support can exert a stabilizing influence on the Ag particles.

The origin of these differences in the stability of small metal nanoparticles on different oxide surfaces is associated with the extra stability offered by the strength of chemical bonding of the metal nanoparticle to the underlying oxide surface (and possibly its metal support when the oxide is thin enough). We have integrated the measured heat versus coverage curves (up to the coverage where the heat reaches the bulk sublimation energy) for each of these three $\text{CeO}_{2-x}(111)$ surfaces, and extracted from that integral the ad-

hesion energies for Ag nanoparticles to these oxide surfaces, by exactly the same procedure outlined in (30), which gave an adhesion energy of 0.3 J/m^2 for Ag on $\text{MgO}(100)$. In all cases, we are assuming a hemispherical shape for the Ag nanoparticles. The results are summarized in Table 1.

These results show that the adhesion energy of Ag nanoparticles to $\text{CeO}_2(111)$ is much larger than to $\text{MgO}(100)$, and that this adhesion energy increases with the extent of reduction of the CeO_2 and when the oxide thickness is only 1 nm (thicknesses of 2, 3, and 4 nm gave the same result). Note that these adhesion energies correlate with the initial adsorption energy of the first Ag gas pulse (Table 1), which corresponds to making clusters of ~ 4 atoms. Both values reflect the strength of Ag-oxide bonding, but the adsorption energy also includes substantial Ag-Ag bonding.

Table 1. Calorimetrically measured adhesion energies of Ag nanoparticles to $\text{MgO}(100)$ and reduced $\text{CeO}_{2-x}(111)$ surfaces, and average Ag particle size and coverage used for these measurements. The adhesion energy for Ag on Ag is given for comparison. Also shown is the initial heat of Ag adsorption (ΔH_{ads}) for the first pulse (~ 0.03 monolayer) of Ag gas.

Surface	Adhesion energy (J/m^2)	Ag coverage (atoms/cm^2)	Particle size (nm)	ΔH_{ads} (kJ/mol)
$\text{MgO}(100)$	0.3 ± 0.3 (30)	9.6×10^{15}	6.6	176
$\text{CeO}_{1.9}(111)$	2.3 ± 0.3	2.8×10^{15}	3.6	200
$\text{CeO}_{1.8}(111)$	2.5 ± 0.3	2.8×10^{15}	3.6	220
$\text{CeO}_{1.9}(111)^*$	2.6 ± 0.3	2.8×10^{15}	3.6	250
Ag(solid)	2.44 (30)	$\infty^\dagger$	$\infty^\dagger$	$285^\ddagger$

*This is a 1-nm film of $\text{CeO}_{1.9}(111)$ on Pt(111). The other oxides are 4-nm films. † Bulk Ag(solid), representing the high-coverage, large-particle limit. ‡ Heat of sublimation of bulk Ag.

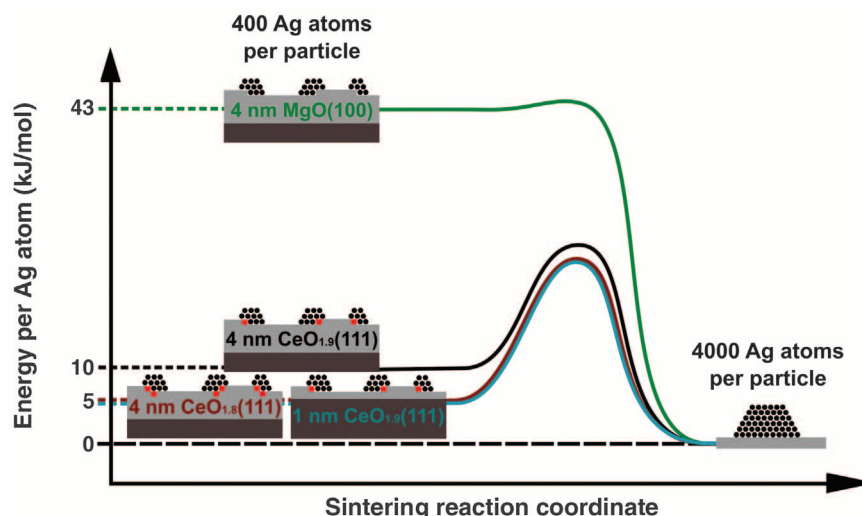


Fig. 3. Schematic representation of the differences in the energetics for the sintering of Ag nanoparticles on the different support surfaces studied here. The thermodynamic driving force for sintering of 400-atom Ag particles (diameter of 3 nm) is much greater on $\text{MgO}(100)$ than on the slightly reduced $\text{CeO}_2(111)$ surfaces. The particles are shown attached to step edges on the basis of prior STM studies of related systems (see text). The red squares represent oxygen vacancies at these steps, and their number reflects the density of these oxygen vacancies. The stability of the Ag atoms approaches their stability in bulk Ag(solid) (the zero energy reference here) by the time they grow to 5000 atoms on all these surfaces, so the structure at the right is meant to represent all four support surfaces. Although not measured here, the activation energies for sintering shown here were estimated based on Brønsted relations: The transition state energy changes proportional to the reaction energy change with a slope of 0.5 (i.e., $\beta = 0.5$). The activation energies change even more than shown, according to our kinetic model (33, 34).

Because it is not possible to create oxygen vacancies in a similar controlled way on this $\text{MgO}(100)$ surface (because of their instability), and because we could not make $\text{CeO}_{2-x}(111)$ with $x < 0.1$, we could not test whether this difference in Ag adsorption and adhesion strength between $\text{CeO}_2(111)$ and $\text{MgO}(100)$ is mainly caused by the residual oxygen vacancies on $\text{CeO}_{1.9}(111)$ or is attributable to some intrinsic difference between $\text{CeO}_2(111)$ and $\text{MgO}(100)$. However, our measurements do reflect faithfully the situation in real catalytic materials under reaction conditions, where MgO exists with very few oxygen vacancies but CeO_2 typically has many vacancies.

Because this large energy difference for Ag particles between CeO_2 and MgO in Fig. 2 extends to 3D particles as large as 1000 atoms, the Ag atoms in such particles are not mainly bonded to oxygen vacancies. Although almost every such Ag particle is likely attached to a few oxygen vacancies at a step edge, a much larger number of Ag atoms are stabilized by interactions with CeO_2 (relative to MgO) than those few Ag atoms that are directly bound to oxygen vacancies on CeO_2 . Thus, this difference must also reflect stronger bonding of Ag to $\text{CeO}_2(111)$ terraces than to $\text{MgO}(100)$ terraces. The adhesion energies of Ag nanoparticles to these $\text{CeO}_{2-x}(111)$ surfaces (~ 2.3 to 2.6 J/m^2) approach and even exceed the adhesion energy of Ag to itself (twice the surface energy of Ag, 2.44 J/m^2) (30). For an adhesion energy equal to or larger than the Ag-Ag adhesion energy, one would generally not expect the Ag to cluster into 3D islands, but instead to wet the surface and form a continuous film. However, these results are for a Ag particle binding locally to some part of the ceria surface where there is likely a much greater defect (step, kink, or vacancy) concentration than elsewhere, and thus the local adhesion energy is likely much less on the stoichiometric CeO_2 terraces. Furthermore, the lattice mismatch between $\text{Ag}(111)$ and the underlying $\text{CeO}_2(111)$ will cause the CeO_2 lattice under the Ag island to contract or expand to gain interfacial bonding stability. These effects would force the ceria lattice immediately adjacent to the island to strain in the opposite direction and also destabilize Ag bonding to the oxide next to the particle.

This lattice strain effect, which is supported by the combined evidence of these adhesion energies and particle morphologies, effectively creates a repulsive interaction between neighboring Ag nanoparticles. Such a repulsion should act like an activation barrier to prevent two Ag nanoparticles from diffusing together and agglomerating, and thus should inhibit catalyst sintering. It also explains the unexpected result that the number density of Ag particles is not larger on the surfaces to which Ag bonds more strongly. These local bonding and local strain effects must be very important in determining the stability of late transition metal nanoparticle catalysts and in understanding metal film morphology, nucleation, and growth during metal deposition onto oxide surfaces.

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Supporting Online Material

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Materials and Methods

Table S1

References

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Evidence of Recent Thrust Faulting on the Moon Revealed by the Lunar Reconnaissance Orbiter Camera

Thomas R. Watters,^{1*} Mark S. Robinson,² Ross A. Beyer,^{3,4} Maria E. Banks,¹ James F. Bell III,⁵ Matthew E. Pritchard,⁶ Harald Hiesinger,^{7,8} Carolyn H. van der Bogert,⁷ Peter C. Thomas,⁹ Elizabeth P. Turtle,¹⁰ Nathan R. Williams⁶

Lunar Reconnaissance Orbiter Camera images reveal previously undetected lobate thrust-fault scarps and associated meter-scale secondary tectonic landforms that include narrow extensional troughs or graben, splay faults, and multiple low-relief terraces. Lobate scarps are among the youngest landforms on the Moon, based on their generally crisp appearance, lack of superposed large-diameter impact craters, and the existence of crosscut small-diameter impact craters. Identification of previously known scarps was limited to high-resolution Apollo Panoramic Camera images confined to the equatorial zone. Fourteen lobate scarps were identified, seven of which are at latitudes greater than $\pm 60^\circ$, indicating that the thrust faults are globally distributed. This detection, coupled with the very young apparent age of the faults, suggests global late-stage contraction of the Moon.

Most large-scale crustal deformation on the Moon is directly associated with the nearside mare-filled basins and is expressed as contractional wrinkle ridges and extensional arcuate and linear rilles or graben (1, 2). Basin-radial and basin-concentric wrinkle ridges occur in the basin interiors, whereas graben are found at basin margins and in adjacent highlands. The stresses that form this pattern of deformation are the result of loading from uncompensated mare basalt fill that induces subsidence and downward flexure of the lithosphere (3). Lobate scarps are tectonic landforms (4–7) that, unlike nearside wrinkle ridges and graben, are generally found outside of mare-filled basins in the highlands and are the most common tectonic landform on the

farside (2). In contrast to basin-related wrinkle ridges and graben, lobate scarps are relatively small-scale structures. They are generally linear or curvilinear asymmetric landforms with relatively steeply sloping scarp faces and are often segmented. Analogous large-scale lobate scarps found on Mercury (8–11) and Mars (12) can have over a kilometer of relief; in contrast, known lunar lobate scarps generally have a maximum relief of <100 m (2, 4–7) and proportionately smaller lengths (less than tens of kilometers) (2, 7). Based on their morphology and crosscutting relations, these structures are interpreted to be contractional landforms resulting from low-angle thrust faulting (4–7, 13). Estimates of the fault displacement-length scaling relations and the linkage between

individual scarp segments further support the interpretation that lobate scarps are the surface expression of shallow thrust faults (2). Although many lobate scarps are found in the highlands, some occur in mare basalts and others transition from lobate scarps to wrinkle ridges (2, 5, 14). Because most previously identified lobate scarps could be easily identified only in high-resolution Apollo Panoramic Camera images (13, 15, 16), covering only a portion of the lunar equatorial zone, their global spatial distribution was unknown. The Lunar Reconnaissance Orbiter Camera (LROC) Narrow Angle Cameras (NACs) and the Wide Angle Camera (WAC) on the Lunar Reconnaissance Orbiter (LRO) have obtained images of known lobate scarps as well as previously undetected scarps ($n = 14$). NAC high-resolution images (0.5 to 2 m per pixel) and topography derived from NAC stereo images allow the most detailed characterization of the morphology and relief of lunar lobate scarps to date.

The Lee-Lincoln scarp ($\sim 20.3^\circ\text{N}$, 30.6°E), just west of the Apollo 17 landing site in the Taurus-Littrow valley, is a well-known lobate scarp (17, 18) that cuts across the mare basalt-filled valley trend-

¹Center for Earth and Planetary Studies, Smithsonian Institution, Washington, DC 20560, USA. ²School of Earth and Space Exploration, Arizona State University, Tempe, AZ 85251, USA. ³Carl Sagan Center, SETI Institute, Mountain View, CA 94043, USA. ⁴NASA Ames Research Center, Moffett Field, CA 94035-0001, USA. ⁵Department of Astronomy, Cornell University, Ithaca, NY 14853, USA. ⁶Department of Earth and Atmospheric Sciences, Cornell University, Ithaca, NY 14853, USA. ⁷Institut für Planetologie, Westfälische Wilhelms-Universität, 48149 Münster, Germany. ⁸Department of Geological Sciences, Brown University, Box 1846, Providence, RI 02912, USA. ⁹Center for Radiophysics and Space Research, Cornell University, Ithaca, NY 14853, USA. ¹⁰Johns Hopkins University Applied Physics Laboratory, Laurel, MD 20723, USA.

*To whom correspondence should be addressed. E-mail: watterst@si.edu

ing roughly north-south between the prominent north and south highland massifs (Fig. 1A). Topography derived from NAC stereo images of the Taurus-Littrow valley (figs. S1 to S3) indicates that the northern segment of the valley-floor scarp has a maximum relief of ~130 m and a narrow low-relief rise associated with the scarp face (figs. S2 and S3). The rise is more pronounced and has greater relief (~20 m) on the southern segment of the valley-floor scarp. The southern segment of the scarp is also flanked by lobate low-relief foothills with a maximum relief of ~40 m (Fig. 1A and fig. S2). NAC images reveal a previously undetected array of narrow shallow troughs in the back-scarp area, west of the scarp face (Fig. 1B). The small-scale troughs have maximum widths of ~25 m and are typically 100 to 200 m in length. Many of the troughs are shallow, with relatively steeply sloping walls and flat floors. We interpret these troughs to be small-scale fractures and graben, indicating extension of the regolith layer and the underlying mare basalts. These graben are among the smallest-scale tectonic landforms yet observed on the Moon. Assuming that the antithetic normal faults of the graben have equal fault-plane dips of ~60° (which is typical for normal faults) and are rooted at the base of the regolith, the regolith depth is estimated to be on the order of the maximum width of the graben. A depth of ~25 m is consistent with the estimated regolith depth at the Apollo 17 landing site (~10 to 32 m), based on regional seismic profile models (19). The orientation of the graben varies from west-northwest to north-east. Thus, some of the graben and fractures are subparallel to the orientation of the scarp, where-

as others are nearly perpendicular to the scarp (Fig. 1B). The most likely explanation for the back-scarp fractures and graben is flexural bending of the valley-floor basalts, where bending stresses cause extension and faulting of the upper regolith layer. Thrust faults are often accompanied by small-scale parasitic faults that result from flexural bending and layer-parallel extension (14, 20).

The Lee-Lincoln fault scarp (Fig. 1A) is not confined to the mare basalts of the Taurus-Littrow valley (4). To the north, the fault cuts across the contact between the valley basalts and the highlands of North Massif, where it extends up-slope for ~400 m before abruptly changing orientation to the northwest, cutting along-slope for over 5 km. The highlands scarp face is about 1 km from the valley floor along much of its length and has a maximum relief of ~5 m (fig. S4).

NAC images of the farside highlands Mandel'shtam scarp (~6.9°N, 161°E), first identified in Apollo Panoramic Camera images (7), show that the scarp face is characteristically lobate (Fig. 2A). Like the Lee-Lincoln scarp, Mandel'shtam has subsidiary scarps that form low-relief foothills along some segments. The north-south-trending scarp consists of several segments with a total length of ~12 km. It is one of a series of scarps that occur in the area near Mandel'shtam crater (7). NAC images show that the northern terminus of Mandel'shtam scarp appears to be made up of a complex series of small scarps (Fig. 2A). These small scarps are interpreted to be the surface expression of splay faults.

So far, 14 previously unknown lobate scarps have been revealed in NAC images (table S1).

These scarps occur in highland material. The northernmost scarp, Rozhdestvenskiy-1, is found on a ledge of rim material along the northern wall of the 42-km-diameter Rozhdestvenskiy crater (~87.5°N, 211.7°E). The east-west-trending scarp has a minimum length of ~5 km and has forefront subsidiary low-relief scarps (Fig. 2B). The Rozhdestvenskiy-1 scarp terminates to the west at the rim of a ~330-m-diameter impact crater. The smallest of the previously unknown lobate scarps is located in the floor material of Slipper crater. This scarp (~48.3°N, 160.5°E) is divided into several segments and is only ~3 km in length (Fig. 2C). The western terminus of the east-west-trending Slipper scarp, like the northern terminus of the Mandel'shtam scarp, consists of a series of splay faults expressed by multiple scarp segments. The vergent side of some of these small fault segments is reversed from south- to north-facing (Fig. 2C). Splay faults are not restricted to the ends of lobate scarps. A previously unknown scarp (~74°S, 8.8°E) located near Simpelius crater has a northeast-trending splay fault that intersects the roughly east-west-trending main scarp at an acute angle (Fig. 2D). The western segment of the Simpelius scarp has multiple terraces that may be the expression of imbricate thrust faulting (Fig. 2D). Multiple terraces suggestive of imbricate faults are associated with other previously known lunar lobate scarps (2). The southernmost previously unknown lobate scarp (~86.3°S, 54.7°E) is found near Shoemaker crater. The northern segment of the north-northwest-trending scarp appears to have relatively high relief and a steeply sloping scarp face (Fig. 2E). The scarp cuts along a roughly NW-SE-oriented linear fabric and across an orthogonal NE-SW-oriented fabric in the regolith.

The majority of the previously known lunar scarps are located in the equatorial zone (Fig. 3). Only 20% of the surface of the Moon was imaged by the Panoramic Cameras. It is likely that less than 10% of this coverage had lighting geometry optimal for detecting the small-scale lobate scarps (2, 7). Of the 14 lobate scarps detected in NAC images, seven occur at latitudes greater than ±60° (Fig. 3 and table S1). Three of these scarps, Shoemaker and Rozhdestvenskiy-1 and -2, are located very near the poles. Apparent gaps in the longitudinal distribution of the scarps detected with the NACs, particularly in the equatorial zone, are probably due to limited image coverage in those regions at the time of the survey. The occurrence of previously undetected lobate scarps at high lunar latitudes, along with the distribution of the mid- and low-latitude scarps on both the near-side and farside, suggests that the thrust faults are globally distributed (Fig. 3).

Lobate scarps appear to be very young, among the youngest tectonic landforms on the Moon (2, 7, 13). NAC images of known and previously unknown scarps reveal crosscutting relations with small-diameter impact craters. The North Massiff segment of Lee-Lincoln scarp crosscuts impact craters with diameters as small as ~7 m (Fig. 4A). Mandel'shtam scarp crosscuts impact

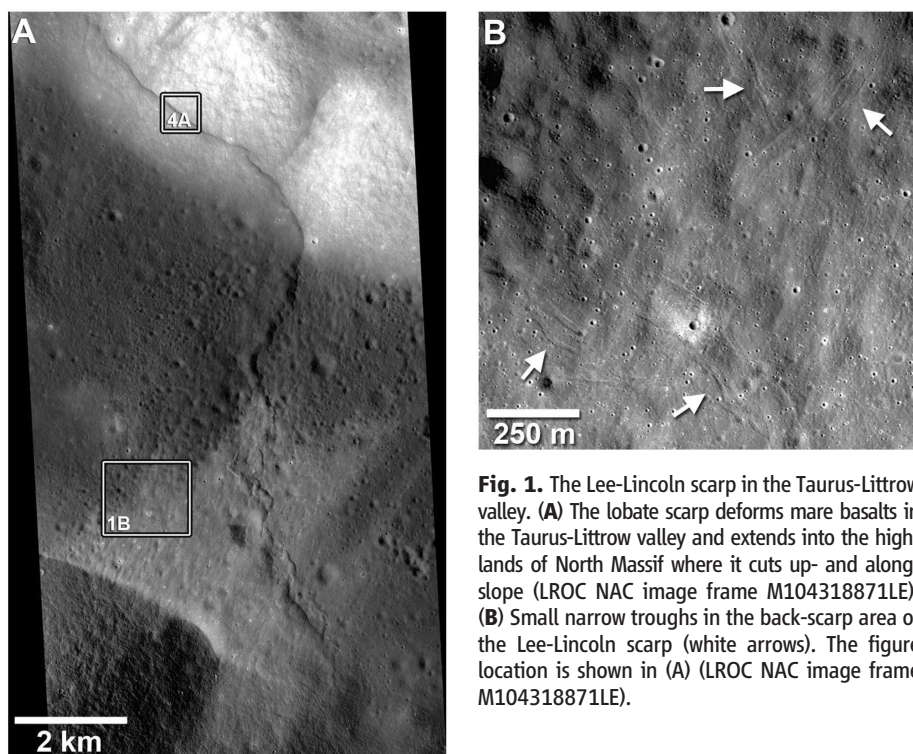


Fig. 1. The Lee-Lincoln scarp in the Taurus-Littrow valley. (A) The lobate scarp deforms mare basalts in the Taurus-Littrow valley and extends into the highlands of North Massif where it cuts up- and along-slope (LROC NAC image frame M104318871LE). (B) Small narrow troughs in the back-scarp area of the Lee-Lincoln scarp (white arrows). The figure location is shown in (A) (LROC NAC image frame M104318871LE).

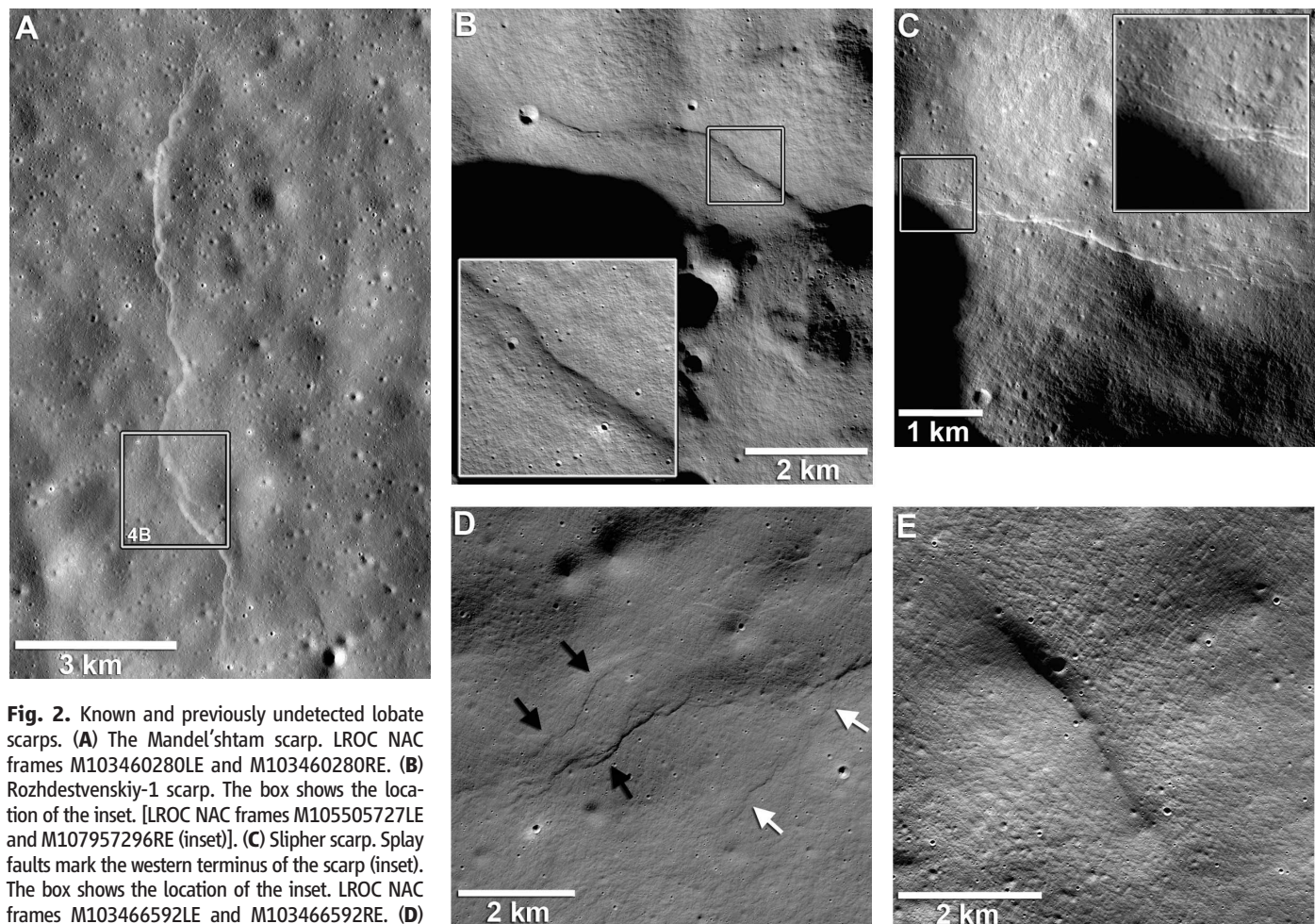
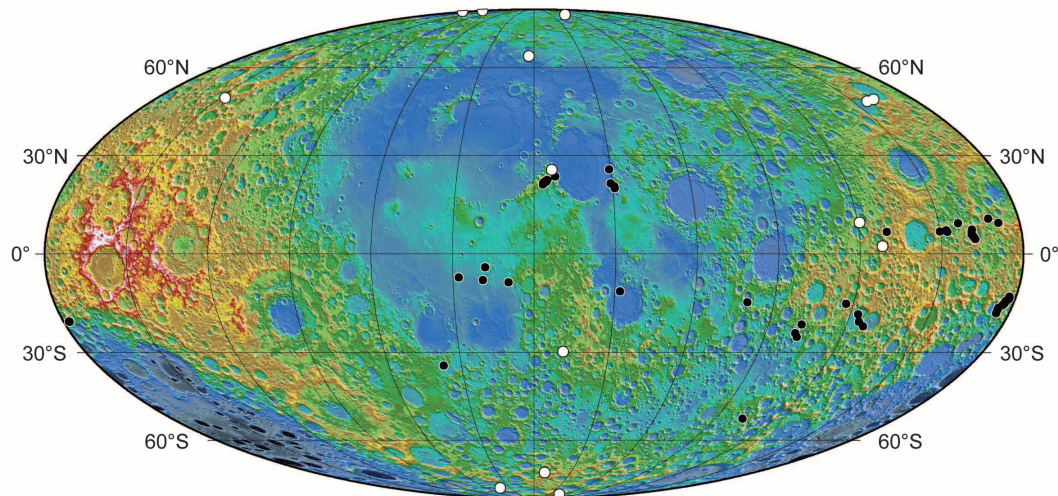


Fig. 2. Known and previously undetected lobate scarps. (A) The Mandel'shtam scarp. LROC NAC frames M103460280LE and M103460280RE. (B) Rozhdestvenskiy-1 scarp. The box shows the location of the inset. [LROC NAC frames M105505727LE and M107957296RE (inset)]. (C) Slipher scarp. Splay faults mark the western terminus of the scarp (inset). The box shows the location of the inset. LROC NAC frames M103466592LE and M103466592RE. (D) Simpelius scarp has a splay fault (white arrows) and multiple fault-controlled terraces (black arrows). LROC NAC frames M106807247LE and M106807247RE. (E) Lobate scarp near Shoemaker crater. LROC NAC frames M108891721LE and M108891721RE.

Fig. 3. The spatial distribution of previously known (black dots) and previously unknown (white dots) lobate scarps on the Moon. The distribution of most of the previously known lobate scarps correlates with the limited Apollo Panoramic Camera coverage of the equatorial region. Lobate scarp locations are plotted on a shaded relief map merged with the Lunar Orbiter Laser Altimeter global 64-pixel-per-degree topographic model.



craters of various scales and with various states of degradation (Fig. 4B). Assigning absolute ages to scarps from crater counts is challenging, because they are structural elements that have a limited spatial extent. An upper bound on the age of the scarp can be estimated based on the ages of the

stratigraphic units containing crosscut craters. The crosscut craters are <50 m in diameter and from estimates of relative ages of lunar craters with various diameters and degrees of degradation, craters 50 m in diameter or smaller are Copernican in age (21). The absolute age of the base

of the Copernican is estimated to be $\sim 800 \pm 15$ million years (if defined by the age of Copernicus crater) (22). Thus, the lunar scarps described here are inferred to be <1 billion years old. This age is consistent with the upper-bound age of lobate scarps estimated by Binder and Gunga (7). The

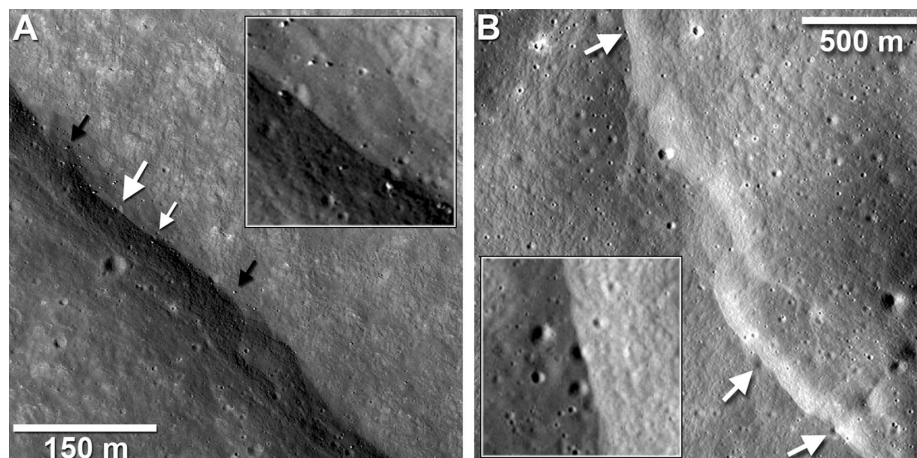


Fig. 4. Crosscutting relations between lobate scarps and impact craters. (A) The Lee-Lincoln scarp in North Massif crosscuts an ~12-m-diameter impact crater (large white arrow and inset) and a ~7-m-diameter crater (small white arrow and inset). Boulders are found along the scarp face (black arrows). The figure location is shown in Fig. 1A. (LROC NAC frame M119652859LE.) (B) Degraded ~40-m-diameter craters (lower white arrows) and a ~20-m-diameter crater (upper white arrow and inset) are crosscut by the Mandel'shtam scarp. The figure location is shown in Fig. 2A. LROC NAC frames M103460280LE and M103460280RE.

most compelling evidence for a very young age for the lobate scarps is their morphologically crisp, undegraded appearance, transected and disturbed meter-scale impact craters, and a lack of superimposed of large-diameter (>400 m) impact craters.

A major constraint on the initial temperature and thermal evolution of the Moon are estimates of radial contraction (23–26). The lack of distributed large-scale lobate scarp thrust faults such as those on Mercury that express significant radial contraction argues against secular cooling of a completely molten early Moon (24, 25). Alternatively, the lack of large-scale thrust faults (scarps >100 km in length and with >500 m of relief) may be due to the accommodation of significant contractional strain by the near-surface regolith and an underlying pervasively fractured zone (27). The relatively young, globally distributed population of small-scale thrust faults described here, however, may be evidence of late-stage radial contraction of the Moon.

Alternatively, substantial tidally induced stresses in the Moon, a tidally locked satellite undergoing orbital recession, may have been generated from relaxation of an early tidal bulge (28). The resulting stresses are expected to cause contraction and thrust faulting in the region around the sub-Earth point and its antipode and extension and normal faulting at the poles (28, 29). This predicted pattern is not consistent with the observed spatial distribution of lobate scarps. Tidal stresses raised solely by Earth are another possible source of global stress; however, their magnitude (a maximum of tens of kilopascals) (30) is probably too low to initiate thrust faulting (31). However, tidal stresses are a likely component of the total stress that formed the thrust faults.

The areal contractional strain estimated with the displacement-length (D - L) scaling relation of

previously known lobate scarps (2), with an updated D - L value for the Lee-Lincoln scarp, is ~0.01% (32). This estimated contractional strain, extrapolated to the entire surface, is equivalent to a radius change of ~100 m and corresponds to isotropic stresses due to radial contraction of <10 MPa (2). The detection of previously unknown lobate scarps at high latitudes is consistent with global extrapolation of the regionally derived contractional strain.

Thermal history models for either a nearly or totally molten early Moon, or an early Moon with an initially hot exterior and magma ocean that maintained a cool interior, predict late-stage compressional stresses in the upper lunar crust and lithosphere (6, 24, 25, 33–35). The initially totally molten model predicts stresses of up to 350 MPa (7, 33). Near-surface compressional stresses of this magnitude might be expected to form a population of thrust-fault scarps comparable in scale to the lobate scarps found on Mercury. Conversely, magma ocean thermal models that limit the change in lunar radius to about ± 1 km in the past 3.8 billion years (since the end of the period of late heavy bombardment) predict compressional stresses of ~100 MPa or less (24, 33). Although a more accurate estimate of the contractional strain expressed by the lobate scarps remains to be determined, the value given here may only be a lower limit if substantial horizontal shortening has not been manifested (27). However, even if the contractional strain is greater by a factor of 2, the compressional stress due to radial contraction is only on the order of ~15 MPa. Thus, in the absence of substantial unexpressed contractional strain, the observations reported here are consistent with thermal history models that predict low-level compressional stresses and relatively small changes (1 km or less) in lunar radius. The relatively young age of the faults suggests that

they formed during a recent episode of lunar radial contraction. Earlier episodes of radial contraction probably resulted in other populations of small-scale thrust faults that are expected to be heavily degraded and as yet unrecognized.

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32. This value of the contractional strain assumes fault-plane dips of 30°. The estimated displacements for eight previously known scarps determined by Watters and Johnson (2) range from ~12 to 108 m (corresponding to a range of horizontal shortening of ~10 to 92 m). The Lee-Lincoln scarp has a displacement of ~260 m (horizontal shortening of ~225 m), estimated with the NAC stereo-derived Digital Terrain Model (figs. S1 and S3).

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36. We thank the three anonymous reviewers for helpful comments that improved the manuscript. We

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5994/936/DC1
SOM Text
Figs. S1 to S4
Table S1

15 March 2010; accepted 8 July 2010
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Drought-Induced Reduction in Global Terrestrial Net Primary Production from 2000 Through 2009

Maosheng Zhao* and Steven W. Running

Terrestrial net primary production (NPP) quantifies the amount of atmospheric carbon fixed by plants and accumulated as biomass. Previous studies have shown that climate constraints were relaxing with increasing temperature and solar radiation, allowing an upward trend in NPP from 1982 through 1999. The past decade (2000 to 2009) has been the warmest since instrumental measurements began, which could imply continued increases in NPP; however, our estimates suggest a reduction in the global NPP of 0.55 petagrams of carbon. Large-scale droughts have reduced regional NPP, and a drying trend in the Southern Hemisphere has decreased NPP in that area, counteracting the increased NPP over the Northern Hemisphere. A continued decline in NPP would not only weaken the terrestrial carbon sink, but it would also intensify future competition between food demand and proposed biofuel production.

Terrestrial ecosystems are a major sink in the global carbon cycle, sequestering carbon and slowing the increasing CO₂ concentration in the atmosphere (1). Terrestrial net primary production (NPP), the initial step of the carbon cycle in which carbon is fixed as biomass, increased from 1982 through 1999, in part due to eased climatic constraints on plant growth (2). The World Meteorological Organization (WMO), National Oceanic and Atmospheric Administration (NOAA), and NASA all reported that 2000 to 2009 was the warmest decade since instrumental measurements of temperatures began in the 1880s (3). We questioned whether the warming climate of the past decade continued to increase NPP, or if different climate constraints were more important.

Between 2000 and 2008, CO₂ emissions from fossil fuel combustion continued to increase at a rate consistent with the average of the highest-emissions family of scenarios, A1FI, used by the Intergovernmental Panel on Climate Change in the Fourth Assessment (1). Carbon-budget methods show that the land is becoming a stronger carbon sink, whereas large uncertainties exist in the partitioning of ocean and land carbon-sink components (1, 4). Satellite data can generally provide realistic information on vegetation dynamics, including land cover change (5, 6), dis-

turbances, and recovery (7), which may help to reduce uncertainties in carbon-budget estimates. In this study, we investigate terrestrial NPP and climate variability over the past decade (2000 to 2009) by analyzing satellite data from the Moderate Resolution Imaging Spectroradiometer (MODIS) on board NASA's Terra satellite and global climate data.

We used the global MODIS NPP algorithm (8) [see supporting online material (SOM) text S1] to examine spatially explicit NPP changes from 2000 through 2009. We used collection 5

(C5) 8-day composite 1-km fraction of photosynthetically active radiation (FPAR) and leaf area index (LAI) data from the MODIS sensor (9) as remotely sensed vegetation property dynamic inputs to the algorithm. Data gaps in the 8-day temporal MODIS FPAR/LAI caused by cloudiness were filled with information from accompanying quality-assessment fields (SOM text S2) (10). For daily meteorological data required to drive the algorithm, we used a reanalysis data set from National Center for Environmental Prediction (NCEP) (SOM text S3) (11). A Palmer Drought Severity Index (PDSI) (12) at 0.5° resolution was used as a surrogate of soil moisture (13) to measure environmental water stress by combining information from both evaporation and precipitation (SOM text S4). A lower PDSI generally implies a drier climate.

Global NPP slightly decreased for the past decade by −0.55 Pg C (Fig. 1). Interannual variations of the global NPP were negatively correlated with the global atmospheric CO₂ growth rates (correlation coefficient $r = -0.89$, $p < 0.0006$) (Fig. 1) (14), suggesting that global terrestrial NPP is a major driver of the interannual CO₂ growth rate. Carbon isotopic measurements have indicated that the exchange of CO₂ with terrestrial ecosystems is the dominant cause of the CO₂ interannual growth rate (15). Though NPP is a part of carbon exchange between the land and atmosphere, the strong correlation may imply that the process of heterotrophic respiration depends ultimately on the substrate supply from NPP (16),

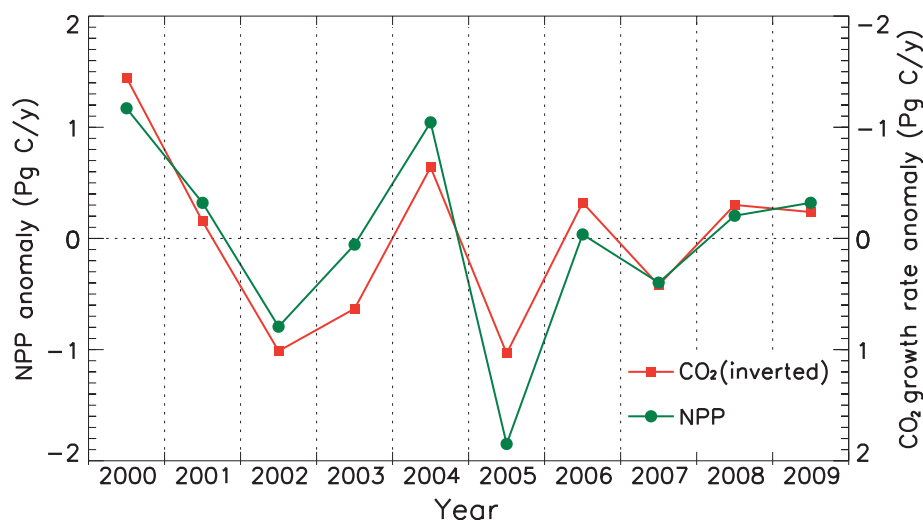


Fig. 1. Interannual variations from 2000 through 2009 in anomalies of annual total global terrestrial NPP (green circles) and inverted global atmospheric CO₂ annual growth rate [red squares and (14)]. Global average annual total NPP is 53.5 Pg C/yr.

Numerical Terradynamic Simulation Group, Department of Ecosystem and Conservation Sciences, the University of Montana, Missoula, MT 59812, USA.

*To whom correspondence should be addressed. E-mail: zhao@nts.g.umn.edu

as shown by the strong correlation between gross primary production (GPP) and ecosystem respiration derived from eddy-flux towers (17). Although also a major contributor, global fire emissions do not correlate as strongly as NPP with interannual CO_2 growth rate (SOM text S5) (18).

Regionally, negative annual NPP anomalies were mainly caused by large-scale droughts (fig. S7 and table S2). In 2000, droughts reduced NPP in North America and China; in 2002, droughts reduced NPP in North America and Australia; in 2003, drought caused by a major heat wave reduced NPP in Europe (19); in 2005, severe droughts in the Amazon (20), Africa, and Australia greatly reduced both regional and global NPP (Fig. 1 and fig. S7); and from 2007 through 2009, over large parts of Australia, continuous droughts reduced continental NPP.

NPP in the tropics (23.5°S to 23.5°N) explains 93% of variations in the global NPP, of

which tropical rainforests explain 61% of global NPP variations (SOM text S8 and table S4). For the three major tropical rainforests (Amazon, Africa and Asia, see fig. S11), Amazon NPP alone explains 66% of the global NPP variations, though it accounts for only 14% of the global total, whereas the other two rainforests have no significant relation with global NPP variations (table S4). The growth of rainforests is mainly constrained by solar radiation due to severe cloudiness (fig. S10) (2, 21), yet our study reveals that, except for rainforests in the islands of southeast Asia, the growth of rainforests in both the Amazon and Africa is also water limited (fig. S10 and table S5). Of the three major rainforests, only Africa had an increasing trend in NPP ($0.189 \text{ Pg C per decade}$), which was mostly due to decreased vapor pressure deficit (VPD) (figs. S9 and S10). The other two had decreasing trends, with the Amazon at $-0.424 \text{ Pg C per decade}$ and Asia at -0.562

Pg C per decade (table S4). The decreased NPP in Asian rainforests was mainly caused by reductions in solar radiation, whereas reduction of NPP in the Amazon was mainly caused by increasing air temperature, which greatly increased autotrophic respiration ($0.38 \text{ Pg C per decade}$), and also by a slight drying trend for the past decade, especially a severe drought in 2005 (figs. S12 and S14 and table S5).

There has been an ongoing debate about whether there was “green-up” of Amazon rainforests in the intense drought of 2005 (22, 23). Hence, we examined MODIS C5 FPAR/LAI, related climate variables, and the resulting GPP and NPP over Amazon rainforests (SOM text S9). FPAR/LAI were higher in 2005, implying green-up of Amazon during drought (figs. S13 and S14). However, annual calculated NPP greatly decreased because of the combined effects of severe water stress and high-temperature-induced high-autotrophic respiration (figs. S13 and S14). The reduced NPP agrees with reported large decreases in plant growth (20). Though there was no green-up of C5 MODIS enhanced vegetation index (EVI) from the drought, there was no clear “browning” either (23). It is unclear what caused green-up of FPAR/LAI or the lack of browning of EVI; however, the reduction in NPP due to severe drought reveals that radiometric greenness is not necessarily a proxy for NPP in some cases. Therefore, remotely sensed NPP models should account for environment stresses and plant autotrophic respiration.

Figure 2 is the spatial pattern of NPP trends over the past decade: NPP increased over large areas in the Northern Hemisphere (NH) while decreasing in the Southern Hemisphere (SH). Over the NH, 65% of vegetated land area had increased NPP, including large areas of North America, Western Europe, India, China, and the Sahel. Regions with decreased NPP include Eastern Europe, central Asia, and high latitudes of west Asia. In the SH, 70% of vegetated land areas had decreased NPP, including large parts of South America, Africa, and Australia.

For northern high latitudes ($>47.5^\circ\text{N}$), warming climate lengthens growing seasons, promoting plant growth (24). However, continuing warming may offset these benefits of an earlier spring and decrease carbon sequestration in a dryer summer and warmer autumn (25, 26). Over the past decade, warming temperatures continued increasing NPP, albeit with a concurrent drying trend (figs. S8 and S9 and table S3). For northern mid-latitudes (22.5°N to 47.5°N), where there are large areas of short-rooted grasslands and croplands (fig. S2), water availability is a dominant control on plant growth. NPP had significant correlations with both growing-season total precipitation ($r = 0.96$, $p < 0.0001$) and average PDSI ($r = 0.76$, $p < 0.05$) (table S3), and a wet trend over the past decade increased NPP (fig. S8 and S9 and table S3). For the Tibet plateau with an average elevation of more than 4500 m, similar to the high latitudes, temperature is the

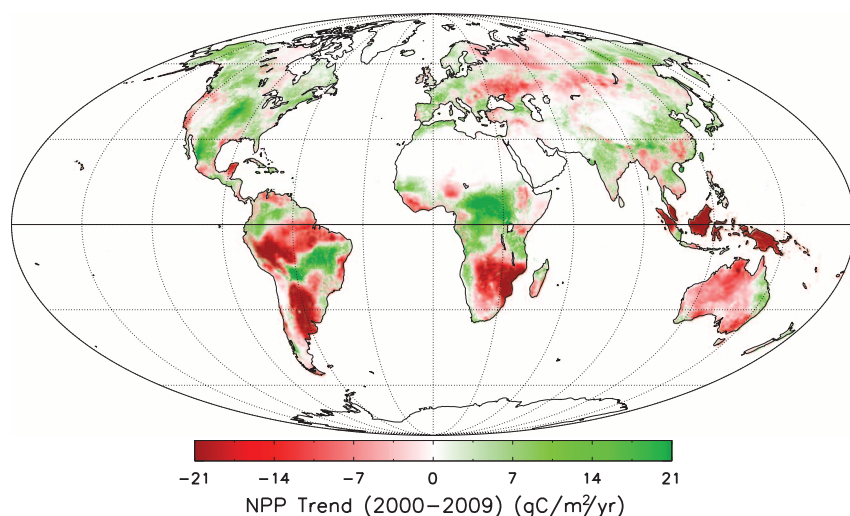


Fig. 2. Spatial pattern of terrestrial NPP linear trends from 2000 through 2009 (SOM text S1) (8, 10).

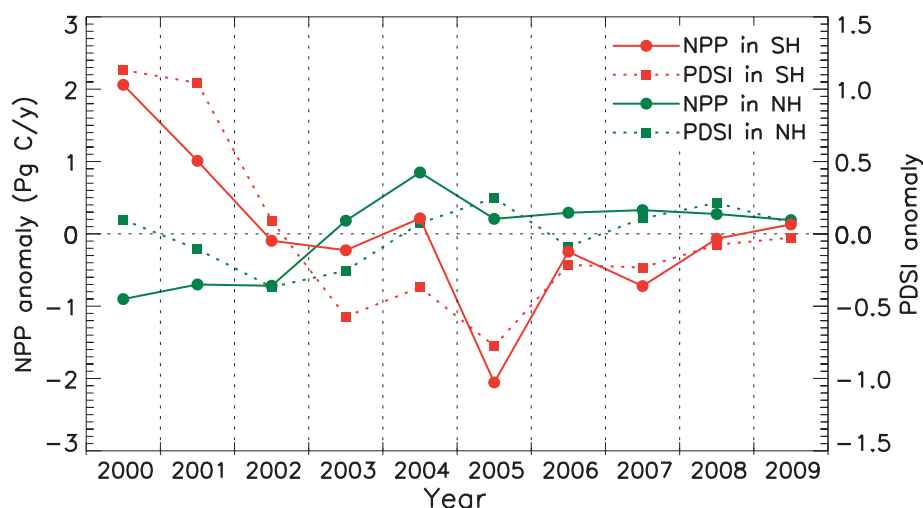
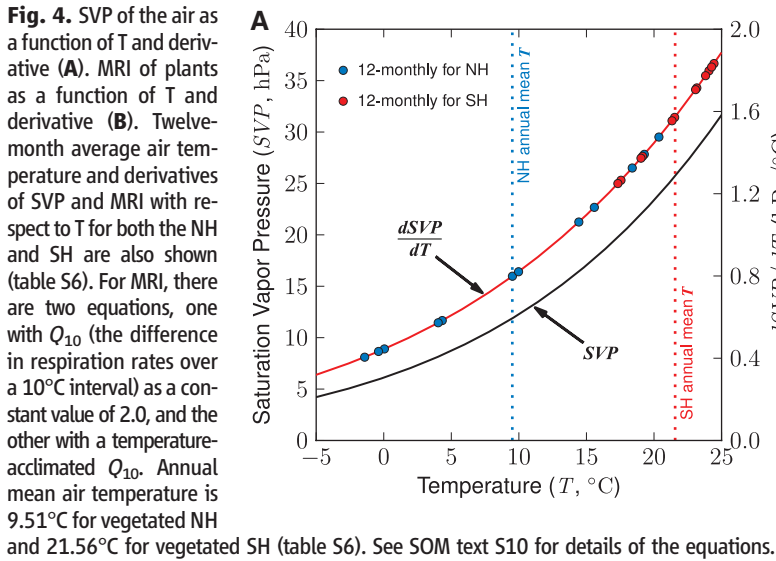


Fig. 3. Interannual variations in total NPP (solid lines) and growing-season average PDSI (dotted lines) over the NH (green) and SH (red) (SOM text S1 and S4).



dominant control on vegetation growth, and the recent warming climate increased NPP (Fig. 2 and figs. S9 and S10). For northern low latitudes (0°N to 22.5°N), reduced atmospheric VPD in Africa and India caused increased NPP in the zone (Fig. 2, fig. S9, and table S3). Overall, the NH had an increasing trend in NPP (Figs. 2 and 3, fig. S8, and table S3). Nitrogen deposition and fertilizer use also contributed to increased NPP in the NH (SOM text S5) (27).

For vegetated land areas in the SH, temperature is not a dominant factor controlling the length of growing season, as indicated by an average of only 7.5 days of snow cover annually, in contrast to 125 days in the NH, as observed by MODIS (fig. S3). Instead, high temperature causes high VPD and evaporation, generally reducing water availability for vegetation growth. Therefore, there is a significant correlation ($r = 0.87$, $p < 0.001$) between NPP and PDSI in the SH, though only a weak correlation ($r = 0.39$, $p < 0.27$) in the NH (Fig. 3). Despite increased precipitation over large parts of the SH (fig. S9 and table S3), the warming trend induced a much higher evaporative demand, leading to a drying trend (fig. S9 and table S3). As a result, NPP decreased because of warming-associated drying trends (Figs. 2 and 3, and figs. S9 and S10). Though NPP in the SH accounts for 41% of the global NPP, the stronger decreasing NPP trend (−1.83 Pg C per decade) counteracted a weaker increasing trend in the NH (1.28 Pg C per decade), resulting in a slight reduction in the global NPP (Figs. 1 and 3 and table S3).

To understand why there are different responses of NPP to a warming climate for two hemispheres, we examine two basic variables governing water and carbon fluxes: (i) saturation vapor pressure (SVP) and (ii) a maintenance respiration index (MRI) (SOM text S10). VPD is the difference between SVP and air vapor pressure, and SVP is a nonlinear function of air temperature (T). VPD is one major factor controlling

surface evapotranspiration (ET) (28); thus, a high T value can increase both VPD and subsequent ET and lead to a dryer environment, eventually reducing NPP. MRI is a power function of T, and a warming climate will increase autotrophic respiration and potentially decrease NPP. Because of the amplification nature of these nonlinear functions to increasing temperature, for different temperature bases, the same increase in air temperature will have much higher increases of SVP and MRI at higher temperature bases than at lower temperatures. Figure 4 shows how SVP and MRI and their derivations respond to air temperatures of the two hemispheres. For vegetated land area, the annual mean T of the SH is more than 12°C higher than that of the NH. An equivalent increase of temperature will consequently have changes in SVP and MRI that are twice as strong in the SH than in the NH. Only during the NH summer (June, July, and August) will there be similar levels of response to warming compared to those of the SH. For the NH, summer is the major growing season; thus, NPP is also susceptible to warming and drought.

Recent simulations from coupled climate–carbon cycle models have shown that there is a positive feedback between the carbon cycle and the climate system (29–31), and also that future biological carbon sinks could eventually level off and subsequently decline to zero (32). Though Fig. 4 only shows MRI responses to temperature, soil respiration largely follows a similar nonlinear temperature response in these models (31). These nonlinear amplification responses of water and carbon processes to warming are the major mechanisms responsible for the positive feedback between the carbon and climate systems.

Though the warming climate during this period continuously increased NPP over areas of high latitude and high elevations, these warming-benefited areas only account for 16% of the global total NPP and 24% of global vegetated land area (SOM text S11). NPP over large land areas

of lower latitude and altitude is negatively correlated with temperature (fig. S10A), mostly due to the warming-related increases in water stress and autotrophic respiration, especially for the SH ($r = -0.94$, $p < 0.0001$). Globally, interannual NPP is negatively correlated with air temperature over vegetated land ($r = -0.64$, $p < 0.05$) (table S3).

Over the past 10 years, large-scale periodic regional droughts and a general drying trend over the SH reduced global terrestrial NPP. Under a changing climate, severe regional droughts have become more frequent, a trend expected to continue for the foreseeable future (13, 33, 34). The warming-associated heat and drought not only decrease NPP, but also may trigger many more ecosystem disturbances (6, 35, 36), releasing carbon to the atmosphere (18, 37). Reduced NPP potentially threatens global food security and future biofuel production and weakens the terrestrial carbon sink. Continuous global monitoring of NPP will be essential to determining whether the reduced NPP over the past 10 years is a decadal variation or a turning point to a declining terrestrial carbon sequestration under changing climate.

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Supporting Online Material

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Figs. S1 to S14

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Loss of DNA Replication Control Is a Potent Inducer of Gene Amplification

Brian M. Green,¹ Kenneth J. Finn,² Joachim J. Li^{3*}

Eukaryotic cells use numerous mechanisms to ensure that no segment of their DNA is inappropriately re-replicated, but the importance of this stringent control on genome stability has not been tested. Here we show that re-replication in *Saccharomyces cerevisiae* can strongly induce the initial step of gene amplification, increasing gene copy number from one to two or more. The resulting amplicons consist of large internal chromosomal segments that are bounded by Ty repetitive elements and are intrachromosomally arrayed at their endogenous locus in direct head-to-tail orientation. These re-replication-induced gene amplifications are mediated by nonallelic homologous recombination between the repetitive elements. We suggest that re-replication may be a contributor to gene copy number changes, which are important in fields such as cancer biology, evolution, and human genetics.

A central tenet of eukaryotic cell biology is that cells replicate their DNA only once every cell cycle. Although it has become an article of faith that this regulation is important because re-replication would threaten genome stability (1), that faith has never been experimentally tested. Preventing re-replication in eukaryotic cells requires blocking re-initiation at thousands of origins scattered throughout the genome, and eukaryotic cells use numerous overlapping mechanisms to do this (2, 3). By disrupting these mechanisms in a controlled manner, we are now able to examine re-replicated cells for possible genomic alterations.

The alterations we first looked for were heritable increases in gene copy number. Such increases are observed in the gene amplifications commonly associated with cancers (4, 5), the gene duplications important for molecular evolution (6), and the copy number variations prevalent in human genomes (7). Although re-replication was once a leading model for copy number increases, specifically during gene amplification (8, 9), the model has long been abandoned for lack of experimental support for it (10). Hence, re-replication is not seriously considered as a possible source of heritable copy number changes (4, 11, 12). Here, we demonstrate that re-replication can readily induce copy number changes, provoking reconsideration of re-replication as a potential source of such changes in cancer and evolution (8).

We have developed a system to detect and quantify early amplification events arising from a transient and limited pulse of re-replication at a defined genomic locus in the budding yeast *Saccharomyces cerevisiae* (Fig. 1A and figs. S1

and S2A). We took advantage of our ability to induce re-replication predominantly from a single origin (*ARS317*) by conditionally deregulating the replication initiation proteins Mcm2-7 and Cdc6 (MC_{2A} genetic background) (13). We also adapted a copy number assay in which cells with a single copy of the *ade3-2p* allele turn pink, and cells with two or more copies turn red (14).

ARS317 and *ade3-2p* were combined in a re-replicating reporter cassette (fig. S1A) that was integrated at either of two loci on chromosome IV (Chr IV_{567kb} and Chr IV_{1089kb}) in a haploid MC_{2A} background from which the endogenous *ARS317* was deleted. After arresting cells at the G₂/M phase [time (*t*) = 0 hours], we transiently induced re-replication until half of the *ARS317* origins re-initiated (*t* = 3 hours), then plated for isolated colonies at both time points. The mostly pink colonies were screened for heritable amplification of *ade3-2p* by looking for colonies with red sectors (fig. S1B). Colonies with one-half, one-quarter, or one-eighth red sectors were scored to focus on amplifications arising within three generations of the re-replication pulse (table S1).

Inducing re-replication for 3 hours from the *ade3-2p-ARS317* cassette integrated at Chr IV_{567kb} (strain YJL6558) caused a 42-fold increase in red sectors to 3.3% of all colonies (Fig. 1B). Suppressing this re-replication by removing deregulated Cdc6 from the MC_{2A} background (YJL6974) or *ARS317* from the cassette (YJL6555) resulted, respectively, in only 4- and 12-fold increases in red sectors (Fig. 1B), most of which had not amplified the *ade3-2p* cassette. Re-replication also induced colony sectoring by 38-fold when the *ade3-2p-ARS317* cassette was relocated to Chr IV_{1089kb} (fig. S2B and table S1).

Array comparative genomic hybridization (aCGH) on 35 red sectors derived from YJL6558 (table S2) confirmed that most [31 of 35 (31/35)] had at least two copies of an internal chromo-

¹Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

²Department of Biochemistry and Biophysics, University of California, San Francisco (UCSF), San Francisco, CA 94158, USA. ³Department of Microbiology and Immunology, UCSF, San Francisco, CA 94158, USA.

*To whom correspondence should be addressed. E-mail: joachim.li@ucsf.edu

somal segment encompassing the *ade3-2p-ARS317* cassette. Amplicons ranged in size from 135 to 470 kb (Fig. 1C) with boundaries mapping within a few kilobases of Ty elements or, rarely, long terminal repeats (LTRs) oriented in direct repeat. Similar aCGH results were obtained for red sectors arising from re-replication of the *ade3-2p* cassette integrated at Chr IV_{1089kb} (fig. S2C and table S3). In contrast, few amplifications

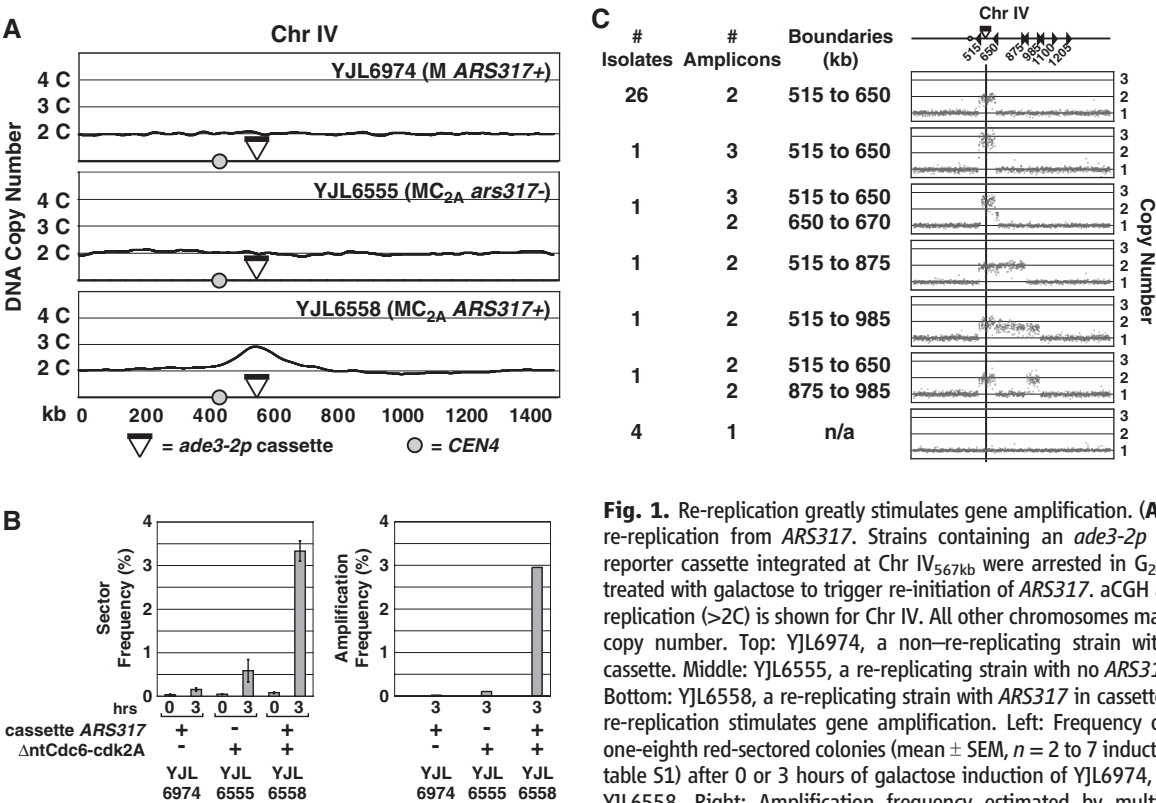


Fig. 1. Re-replication greatly stimulates gene amplification. (A) Induction of re-replication from *ARS317*. Strains containing an *ade3-2p* copy number reporter cassette integrated at Chr IV_{567kb} were arrested in G₂/M phase and treated with galactose to trigger re-initiation of *ARS317*. aCGH analysis of re-replication (>2C) is shown for Chr IV. All other chromosomes maintained a 2C copy number. Top: YJL6974, a non-re-replicating strain with *ARS317* in cassette. Middle: YJL6555, a re-replicating strain with no *ARS317* in cassette. Bottom: YJL6558, a re-replicating strain with *ARS317* in cassette. (B) *ARS317* re-replication stimulates gene amplification. Left: Frequency of one-half to one-eighth red-sectored colonies (mean $\pm$ SEM, $n = 2$ to 7 induction replicates; table S1) after 0 or 3 hours of galactose induction of YJL6974, YJL6555, and YJL6558. Right: Amplification frequency estimated by multiplying sector frequency by the fraction of sectored colonies displaying reporter cassette amplification (tables S2 and S4). (C) Red sectors induced by re-replication display gene amplifications. Thirty-five red sectors derived from YJL6558 were analyzed by aCGH and classified on the basis of the copy number profile of Chr IV. A schematic of Chr IV (top) shows the positions of Ty elements (triangles), the centromere (circle), and the *ade3-2p* cassette (black bar).

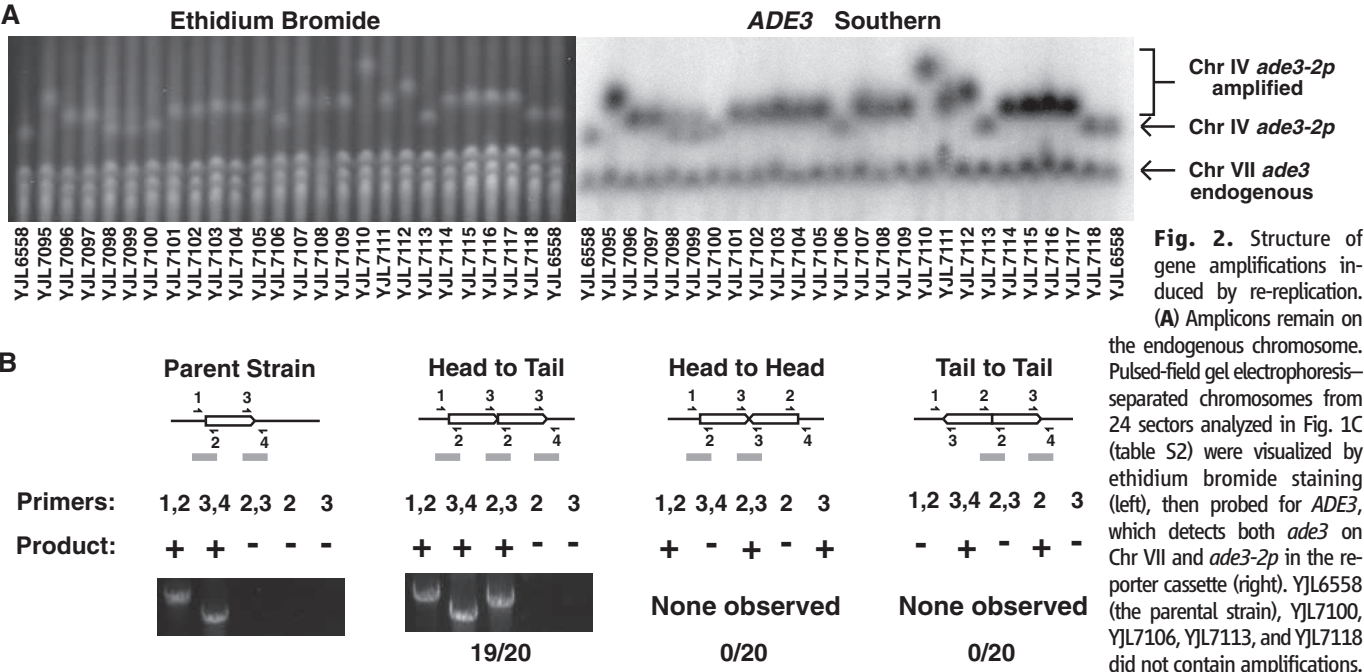


Fig. 2. Structure of gene amplifications induced by re-replication. (A) Amplicons remain on the endogenous chromosome. Pulsed-field gel electrophoresis-separated chromosomes from 24 sectors analyzed in Fig. 1C (table S2) were visualized by ethidium bromide staining (left), then probed for *ADE3*, which detects both *ade3* on Chr VII and *ade3-2p* in the reporter cassette (right). YJL6558 (the parental strain), YJL7100, YJL7106, YJL7113, and YJL7118 did not contain amplifications. (B) Amplicons are tandemly arrayed in loco in direct head-to-tail orientation. Schematics of an unamplified amplicon and three possible orientations for amplicons tandemly duplicated in loco are shown. Predicted PCR junction fragments (10) are shown for five sets of primers that flank amplicon boundaries (+, PCR product expected; -, no PCR product expected). Representative PCR products are shown for parental strain YJL6558 and 19 of the 20 amplified strains displayed in (A).

were observed among the less frequent red sectors isolated from the control strains YJL6974 (3/32) (table S4) and YJL6555 (1/6).

Using the aCGH data to convert sectoring to amplification frequency (10), we estimated relative amplification frequencies of 1:8:230 for YJL6974:YJL6555:YJL6558, respectively, and an absolute frequency of 3×10^{-2} for YJL6558 (Fig. 1B and table S2). This frequency roughly translates into an order of magnitude rate of 10^{-2} per generation (10), which is significantly higher than spontaneous rates of segmental duplications (10^{-7} to 10^{-6} per generation) or higher-order amplifications (10^{-10}) reported for budding yeast (15, 16).

Re-replication generates slowed or stalled forks and DNA damage (17–20), perturbations that have been implicated in genomic alterations (21). Nonetheless, neither disruption of DNA replication by means of hydroxyurea or temperature-sensitive replication mutations (fig. S3, A and B) nor treatment of cells with the DNA-damaging agent phleomycin (fig. S3, C to E, and table S5) induced substantial *ade3-2p* amplification comparable to re-replication. Thus, re-replication appears to be particularly effective at inducing gene amplification (10), and we refer to these events as re-replication-induced gene amplification (RRIGA).

To investigate the mechanism of RRIGA, we determined the position, orientation, and boundaries of 20 segmental amplifications arising from re-replication of the *ade3-2p* cassette integrated

at Chr IV₅₆₇. All 20 were located to Chr IV, because this chromosome increased in size by an amount consistent with the length and copy number of the additional amplicon(s) (Fig. 2A and table S1). No other chromosomes increased in size, and probing for the *ADE3* sequences on the *ade3-2p* cassette confirmed that the amplicons resided only on Chr IV (Fig. 2A).

We then tested the hypothesis that the amplicons were tandemly arrayed at their endogenous locus. The three possible arrangements for such tandem duplications (head to tail, head to head, or tail to tail) each generate a particular set of junctions and boundaries that are distinguishable by polymerase chain reaction (PCR) (Fig. 2B). Moreover, we could confirm the presence of Ty or LTR elements at these boundaries by using primers that flank these elements. Of the 20 amplifications examined, PCR products from 19 of them established that RRIGA amplicons are indeed tandemly arrayed at their endogenous locus in head-to-tail orientation and are bounded by Ty or LTR elements in direct repeat (Fig. 2B) (10).

Such structures could arise from nonallelic homologous recombination (NAHR). To examine this possibility, we sequenced the PCR products for interamplicon junctions from four independent amplifications spanning kilobases 515 to 650 on Chr IV. The sequences revealed hybrid Ty elements generated by precise crossovers between Ty2-1 at 515 kb and Ty1-1 at 650 kb (Fig. 3A and fig. S4). In addition, RRIGA was greatly reduced

by deletion of *RAD52*, which is essential for homologous recombination (Fig. 3, B and C, and tables S1 and S6), but was not affected by deletion of *DNL4*, which is required for nonhomologous end-joining (NHEJ). Thus, RRIGA in budding yeast is mediated by NAHR between repetitive elements flanking a re-replicated chromosomal segment.

How re-replication might stimulate NAHR with such high efficiency is illustrated in Fig. 3D. First, re-replication increases the copy number of a chromosome segment. Second, re-replication forks, which display compromised progression, may stall, collapse, and break with high frequency. Third, in contrast to stalled replication forks in S phase, isolated re-replication forks are unlikely to be rescued by converging forks from neighboring origins. Fourth, the re-replication bubble structure can facilitate recombinational repair between re-replicated segments in a variety of ways, such as by pairing double-stranded breaks that might occur at both forks in trans.

In short, re-replication appears capable of promoting several key events in an optimal temporal order and spatial context to stimulate gene amplifications. The critical events occur before repair pathways are recruited, leaving room for alternative ways to resolve broken re-replication bubbles (10). Hence, although RRIGA is preferentially mediated by NAHR in budding yeast, additional repair pathways, such as NHEJ, might be used by other species. In fact, repair of broken

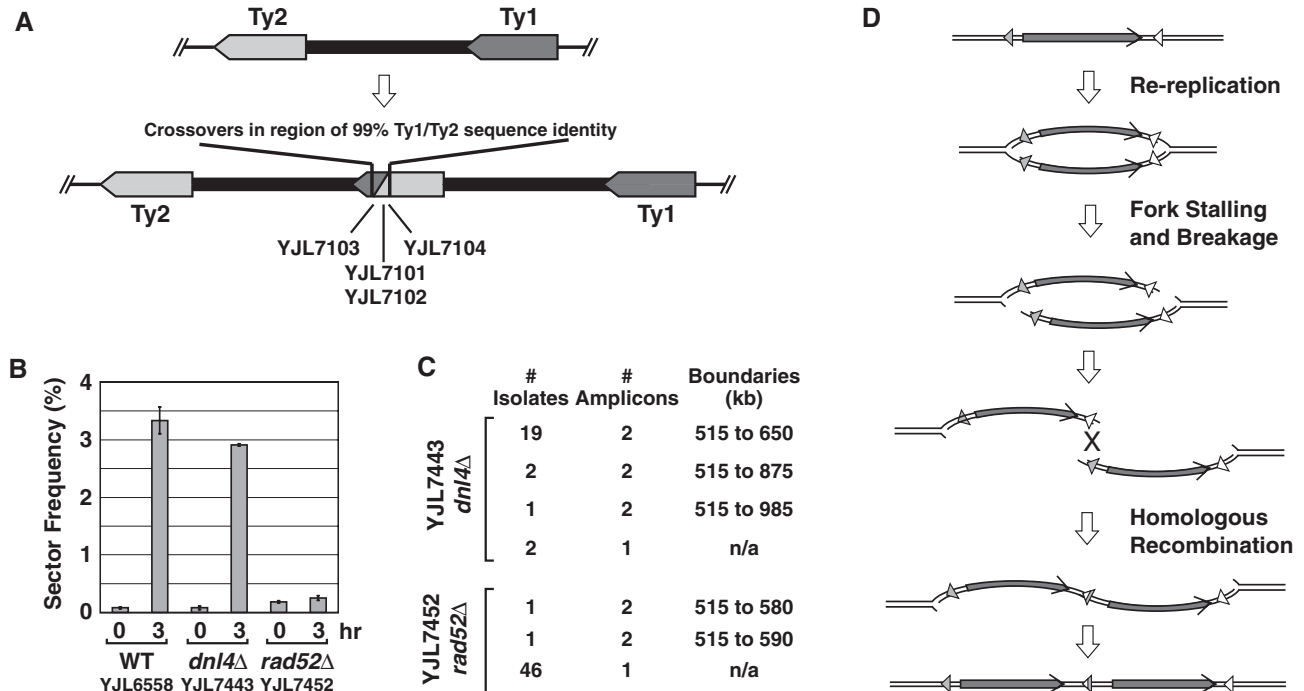


Fig. 3. Role of nonallelic homologous recombination in RRIGA. (A) Schematic of hybrid recombinant Ty elements (fig. S4) found at the interamplicon junction of four isolates with segmental amplifications on Chr IV from 515 to 650 kb. (B) Re-replication-induced sectoring is dependent on HR and not NHEJ. YJL7443 (*dnl4Δ*) and YJL7452 (*rad52Δ*) are otherwise isogenic with re-replicating strain YJL6558. Re-replication-induced sectoring frequency (mean $\pm$ SEM, $n = 2$ to 7

induction replicates; table S1) was analyzed as described for Fig. 1B. (C) Re-replication-induced gene amplification is dependent on HR and not NHEJ. A representative aCGH analysis of Chr IV for 24 red sectors derived from YJL7443 (*dnl4Δ*) and 48 derived from YJL7452 (*rad52Δ*) is shown (table S6). (D) How re-replication might stimulate NAHR. Arrowheads, nonallelic or hybrid recombinant repetitive element; arrows, amplified segments.

re-replication bubbles may well stimulate other genomic alterations besides RRIGA.

Our results demonstrate that loss of eukaryotic DNA replication control can indeed induce genome instability, and in the case of RRIGA does so with extraordinary efficiency. Such efficiency suggests that even lower levels of re-replication, below the sensitivity of current assays (<5 to 10%), may cause substantial induction of RRIGA (10). Thus, although some consider the multiple mechanisms used to control replication as redundant, because disrupting them individually does not lead to detectable re-replication (2), we view each as essential for the stringent control needed to safeguard genome stability (2, 3, 13).

Establishing that re-replication can induce copy number changes raises the question of whether re-replication actually does induce such changes in cancer and evolution (10). Several recent observations hint that RRIGA might indeed play a role in oncogenesis or tumor progression. For example, segmental duplications found in two tumor genomes bear striking similarity to RRIGA structures observed in budding yeast: Oncogenes are duplicated in a head-to-tail arrangement in loco, with repetitive Alu elements at the outer amplicon boundaries and a hybrid recombinant Alu element at the interamplicon junction (22, 23). Additionally, replication initiation proteins are overexpressed in a number of human cancers (1, 24), and modest overexpression of the replication proteins Cdt1 and Cdc6 can potentiate oncogenesis in mouse cells (25–27). We thus hope that our study provokes further investiga-

tion into the possible role of RRIGA in cancer and evolution.

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Supporting Online Material

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The *Legionella* Effector Protein DrrA AMPylates the Membrane Traffic Regulator Rab1b

Matthias P. Müller,* Heide Peters,* Julia Blümer, Wulf Blankenfeldt,† Roger S. Goody,† Aymelt Itzen†

In the course of Legionnaires' disease, the bacterium *Legionella pneumophila* affects the intracellular vesicular trafficking of infected eukaryotic cells by recruiting the small guanosine triphosphatase (GTPase) Rab1 to the cytosolic face of the *Legionella*-containing vacuole. In order to accomplish this, the *Legionella* protein DrrA contains a specific guanine nucleotide exchange activity for Rab1 activation that exchanges guanosine triphosphate (GTP) for guanosine diphosphate on Rab1. We found that the amino-terminal domain of DrrA possesses adenosine monophosphorylation (AMPylation) activity toward the switch II region of Rab1b, leading to posttranslational covalent modification of tyrosine 77. AMPylation of switch II by DrrA restricts the access of GTPase activating proteins, thereby rendering Rab1b constitutively active.

Legionella pneumophila is a human pathogen that creates a replication vacuole in host cells (1). It interferes with normal intracellular vesicular transport by several mechanisms, one of which involves recruitment of the small guanosine triphosphatase (GTPase) Rab1 to the *Legionella*-containing vacuole (LCV) (2, 3).

The bacterial protein DrrA (also known as SidM), which acts as a guanosine triphosphate–guanosine diphosphate (GTP–GDP) exchange factor (GEF) for the GTPase, plays a major role in this process. It has been suggested that DrrA also has GDF (RabGDI displacement factor) activity to displace GDP-bound Rab1 from guanine nucle-

otide dissociation inhibitor (GDI) (4, 5) but this activity merely represents a passive one arising from the GEF activity (6). In addition to the GEF domain, DrrA has at least two further domains (Fig. 1A), one of which is a C-terminal lipid phosphatidylinositol-4-phosphate binding domain (P4M) responsible for membrane attachment (7) and an N-terminal region of hitherto unknown function.

It has been reported that the N-terminal region has cytotoxic properties (4), but the amino acid sequence does not suggest its function. We crystallized the N-terminal region by using a tryptic fragment encompassing amino acids 9 to 218 (DrrA₉₋₂₁₈) and determined its structure at a resolution of 2.1 Å (Fig. 1B and table S1) (8). We observed structural similarities to nucleotidyl transferases, the closest agreement being between DrrA₉₋₂₁₈ and the C-terminal domain of glutamine synthetase adenylyl transferase (GS-ATase) (Fig. 1C) (9). Superimposition with GS-ATase

Department of Physical Biochemistry, Max Planck Institute of Molecular Physiology, Dortmund, NRW, 44227, Germany.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: aymelt.itzen@mpi-dortmund.mpg.de (A.I.); roger.goody@mpi-dortmund.mpg.de (R.S.G.); wulf.blankenfeldt@mpi-dortmund.mpg.de (W.B.)

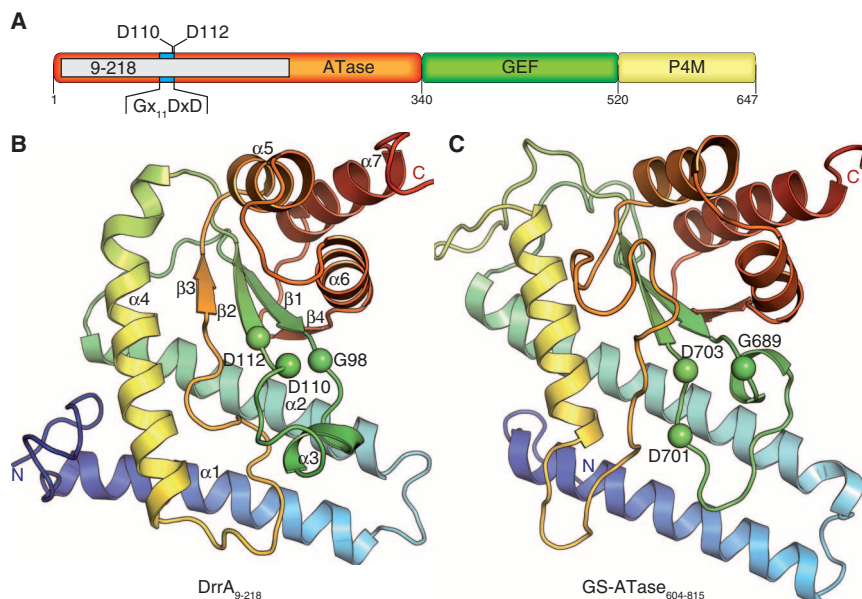


Fig. 1. DrrA₉₋₂₁₈ is reminiscent of nucleotidyl transferases. **(A)** Scheme of the domain architecture of DrrA, indicating the relative positions of the ATase, GEF, and P4M domains and the crystallized fragment DrrA₉₋₂₁₈. **(B and C)** A comparison between the crystal structures of the DrrA₉₋₂₁₈ fragment **(B)** and GS-ATase **(9)** (residues 604 to 815) **(C)**. The catalytically important amino acids of the G-X₁₁-D-X-D motif of GS-ATase and their counterparts in DrrA₉₋₂₁₈ are indicated as green spheres. N and C indicate N and C terminus, respectively.

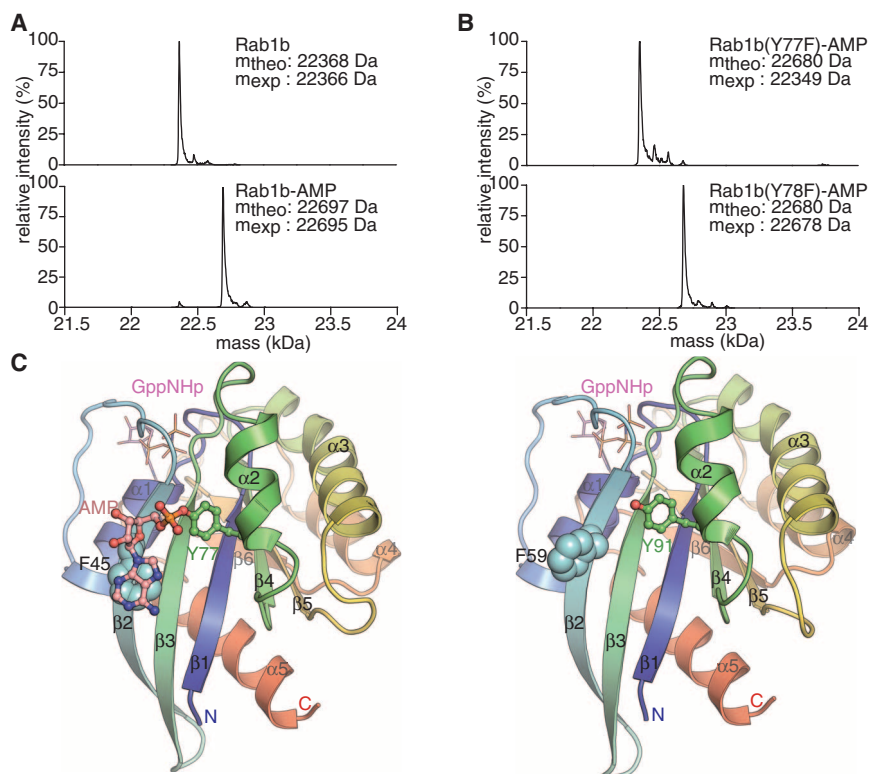


Fig. 2. DrrA specifically AMPylates Y77 of Rab1b. **(A)** AMPylation of Rab1b by DrrA_{fl} was monitored by electrospray ionization mass spectrometry (ESI-MS). AMPylation shifts the molecular weight (*m*) of Rab1b (top) by 329 dalton (Da) (bottom). **(B)** Test for AMPylation of Rab1b point mutants Y77→F77 (Y77F) and Y78F with DrrA_{fl} by ESI-MS, demonstrating the importance of Y77 for AMPylation. **(C)** Structures of Rab1b₃₋₁₇₄-AMP:GppNHp (left) and Rab3a (right). GppNHp is shown as lines; tyrosine and tyrosine-AMP, as balls and sticks, Phe45 (Rab1b) or Phe59 (Rab3a), as spheres.

revealed that DrrA shares the catalytically important sequence motif G-X₁₁-D-X-D (10), which occupies the same position and relative orientation as in GS-ATase.

We thus speculated that the N-terminal region might have adenosine monophosphate (AMP)–transferring activity toward Rab1b, the substrate of its GEF domain, which we confirmed on mixing full-length DrrA (DrrA_{fl}) and Rab1b with adenosine triphosphate (ATP) (Fig. 2A). This activity, which has recently been termed AMPylation (11), appeared to require the full region N-terminal to the GEF domain (DrrA₃₄₀₋₅₃₃) of DrrA because the fragment DrrA₁₋₃₃₉ was catalytically active, whereas shorter constructs were unable to AMPylate Rab1b in vitro (fig. S1 and table S2). Mutating the aspartates of the G-X₁₁-D-X-D motif to alanines (D110/112A) eliminated the enzymatic activity (fig. S1), consistent with the importance of this motif for the catalysis (12). In addition to ATP, GTP was also a substrate for the nucleotide transfer reaction, leading to GMPylation, although ATP appears to be the preferred substrate (fig. S2). Additionally, Rab1b bound to the nonhydrolyzable GTP analog GppNHp was the preferred substrate, because the apparent specific enzymatic activity of DrrA was 6.02 ± 0.23 $\mu\text{moles}_{\text{product}}/\text{mg}_{\text{enzyme}}$ per min for Rab1b:GppNHp versus 0.022 ± 0.001 $\mu\text{moles}_{\text{product}}/\text{mg}_{\text{enzyme}}$ per min for Rab1b:GDP (fig. S3).

In order to determine the site of modification on Rab1b, we performed tandem mass spectrometric analysis on a total tryptic digest of preparatively AMPylated Rab1b (fig. S4). The tryptic peptide $_{72}\text{TITSSYYR}_{79}$ (10) from switch II revealed a mass shift consistent with AMPylation, and the site of modification was mapped to Tyr⁷⁷ (Y77). This was confirmed by using the phenylalanine mutants Rab1b Y77F and Rab1b Y78F: Rab1b Y78F was AMPylated by DrrA₁₋₃₃₉, whereas Rab1b Y77F was not (Fig. 2B). To examine the influence of Y77 modification on Rab1b, we determined the crystal structure of Rab1b-AMP in complex with GppNHp (Fig. 2C, fig. S5, and table S1) and compared it to the structure of the closely related Rab3A (Fig. 2C) (13). AMPylation of Y77 did not significantly alter the overall conformation of the Rab protein. The modified Y77 essentially adopted the same orientation as in the unmodified state with the AMP group close to the surface of the protein.

Because Y77 is mostly conserved in the Rab family, we investigated the AMPylation activity of DrrA toward a variety of recombinant Rab proteins purified from *Escherichia coli* (fig. S6). Rab1b, Rab3a, Rab4b, Rab6a, Rab8a, Rab11a, Rab13, Rab14, and Rab37 served as DrrA_{fl} substrates, whereas Rab5a, Rab7a, Rab9a, Rab22a, Rab23, Rab27a, Rab31, Rab32, and Rab38 did not. AMPylation of other Rab proteins may not play an important role because the membrane localization of DrrA will restrict its AMPylation activity to Rab1b, whose localization is in turn governed by DrrA GEF activity.

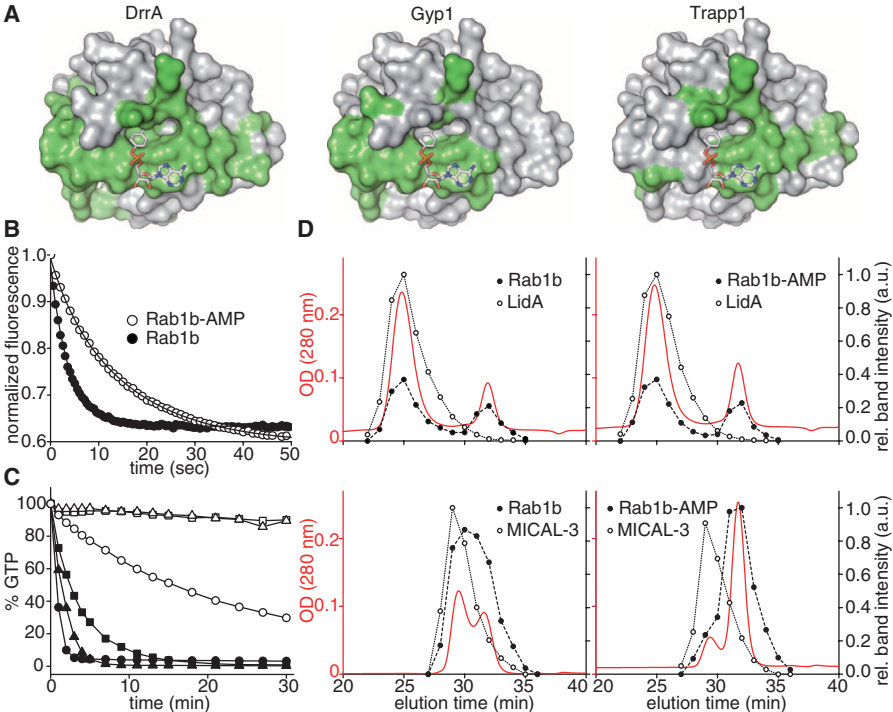


Fig. 3. Biochemical consequences of Rab1b AMPylation. **(A)** The positions of DrrA₃₄₀₋₅₃₃ and Trapp1 interacting residues and the presumable binding residues for Gyp1 and TBC1D20 [inferred from the Rab33:Gyp1-complex structure (14)] are indicated in green in the surface representation of Rab1b-AMP:GppNHp. The AMPylated tyrosine of Rab1b-AMP is shown in stick representation. **(B)** Nucleotide exchange catalyzed by DrrA₃₄₀₋₅₃₃ (1 μM) on 50 nM Rab1b (solid circles) or Rab1b-AMP monitored (open circles) by fluorescence decrease upon release of mantdGDP, fitted to single exponentials. **(C)** GAP-stimulated GTP hydrolysis of 40 μM Rab1b:GTP (solid symbols) and Rab1b-AMP:GTP (open symbols) using 100 nM TBC1D20₁₋₃₆₂ (circles), Gyp1 (squares), or LepB (triangles). The GTP content of individual samples from each time point was quantified by reversed-phase high-performance liquid chromatography. **(D)** Influence of Rab1b-AMPylation on effector binding analyzed by gel filtration. Mixtures of GppNHp-bound Rab1b or Rab1b-AMP with LidA or MICAL-3 were separated by gel filtration (red line), and complex formation was checked by denaturing polyacrylamide gel electrophoresis of individual fractions (black lines) (Rab1b and Rab1b-AMP, solid circles; LidA and MICAL-3, open circles). OD, optical density; a.u., arbitrary units.

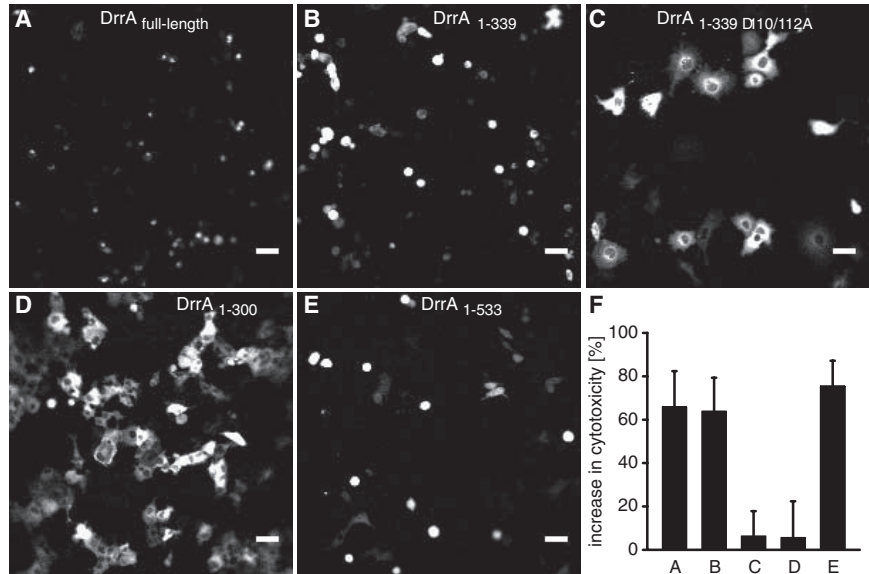


Fig. 4. Cytotoxicity of DrrA is linked to its AMPylation activity. **(A to E)** EGFP fluorescence microscopy images of COS-7 cells transfected with indicated DrrA constructs showing cell rounding and growth defects caused by AMPylation activity of DrrA (scale bars, 50 μm). **(F)** Flow cytometric quantification of COS-7 cytotoxicity resulting from DrrA AMPylation activity. The y axis indicates the increase in cytotoxicity (as determined by propidium iodide staining) of DrrA-expressing cells (EGFP-positive) in relation to the EGFP-vector control. The x axis labels correspond to (A) to (E). Data are mean ± SD from *N* = 4 independent experiments.

The switch II region of Rab1b (in which Y77 is centrally located) is involved in binding to effector and regulator proteins, such as GTPase-activating proteins [GAPs: Gyp1, LepB, and TBC1D20 (14–16)], the tethering factor Trapp1 (17), and the GEF domain of DrrA (DrrA₃₄₀₋₅₃₃) (6) (Fig. 3A). To understand the consequences of Rab AMPylation, we performed *in vitro* interaction experiments with Rab1b-interacting molecules. The GEF reaction of Rab1b-AMP with DrrA₃₄₀₋₅₃₃ was only moderately affected compared with Rab1b (Fig. 3B), whereas the stimulation of GTP hydrolysis by the GAPs Gyp1-46, TBC1D20₁₋₃₆₂, and the *Legionella* protein LepB was strongly inhibited (Fig. 3C). Thus, AMPylation appears to prolong the lifetime of the GTP state of Rab1b by restricting the access of GAPs to switch II and blocking GAP-stimulated GTP hydrolysis. This is also true for GMPylation of Rab1b (fig. S7).

Rab proteins interact with effector proteins in the GTP state, and we asked whether this is still possible for AMPylated Rab1b. The binding of GppNHp-loaded Rab1b and Rab1b-AMP was tested with the mammalian effector MICAL-3 (18) and the *Legionella* effector LidA (5) by using a gel filtration-based assay (Fig. 3D). LidA was able to interact with both Rab1b and Rab1b-AMP, whereas MICAL-3 binding was inhibited by AMPylation. Thus, AMPylation of Rab1b appears to disrupt binding to the mammalian effector protein MICAL-3 but not to the Rab1b effector LidA provided by *Legionella*.

The N-terminal domain of DrrA causes cytotoxicity in mammalian cells (4). In order to test whether this effect is linked to the AMPylation activity of DrrA, we expressed various DrrA constructs N-terminally tagged to the fluorescent reporter enhanced green fluorescent protein (EGFP) in COS-7 cells. DrrA constructs containing the active AMPylation domain (DrrA_{fl}, DrrA₁₋₅₃₃, or DrrA₁₋₃₃₉) caused severe morphological changes of the cells characterized by cell rounding and shrinkage (Fig. 4, A, B, and E). However, more extensively truncated constructs or amino acid point mutations affecting the AMPylation activity of DrrA (DrrA₁₋₃₀₀ and DrrA₁₋₃₃₉ D110/112A) did not have any effect on cell morphology (Fig. 4C, D). Flow cytometry analysis demonstrated a substantial increase in cell death (by about 60%) when the AMPylation activity was present (DrrA_{fl}, DrrA₁₋₅₃₃, and DrrA₁₋₃₃₉), whereas AMPylation-inactive mutants (e.g., DrrA₁₋₃₃₉ D110/112A) were not significantly toxic (Fig. 4F). The cotransfection of DrrA₁₋₃₃₉ constructs with Rab1b Y77F or Rab1b showed a tendency to overcome the cytotoxic effect (fig. S8), indicating that AMPylation of Rab1b could be the cause of cytotoxicity. Thus, the AMPylation activity of DrrA affects cell homeostasis of mammalian cells and causes cytotoxicity.

Rab1 is effectively removed from the LCV about 4 hours after infection (15). This removal probably requires extraction by RabGDI, and, because the interaction with RabGDI is only

possible in the inactive GDP-bound state (20), Rab1 must first be deactivated to be released from the LCV. The probable GAP for this deactivation is the *Legionella* protein LepB because of its specificity for Rab1 and the coincidence of LepB-positive with Rab1/DrrA-negative LCVs (15). However, because LepB is unable to stimulate GTP hydrolysis on AMPylated Rab1b, it seems likely that Rab1b-AMP needs to be de-AMPylated first, immediately raising the question of an unidentified host- or *Legionella*-derived de-AMPylase.

AMPylation of Rab1b by DrrA should be mainly restricted to the LCV membrane, because DrrA specifically localizes here via its interaction with phosphatidylinositol-4-phosphate immediately after infection (7). On the other hand, the localization of LepB to the LCV is delayed with respect to DrrA and Rab1 (15), which could mean that LepB initially acts on Rab1 elsewhere but not at the LCV. This might inhibit intrinsic Rab1-dependent vesicular transport to the Golgi apparatus.

AMPylation of proteins is a mechanism for modulating their enzymatic activity (19, 20) or their interaction with target molecules, in the case of Rho proteins (11, 21). In the latter case, a different, unrelated AMPylation domain (the fic domain) (22) is responsible for the modification. The unexpected finding of DrrA-mediated AMPylation of the switch II region

of Rab1b further adds to our knowledge of this emerging field as a mechanism in signal transduction.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1192276/DC1
Materials and Methods
Figs. S1 to S8
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References and Notes

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Chloroplasts Divide by Contraction of a Bundle of Nanofilaments Consisting of Polyglucan

Yamato Yoshida,¹ Haruko Kuroiwa,¹ Osami Misumi,¹ Masaki Yoshida,¹ Mio Ohnuma,¹ Takayuki Fujiwara,¹ Fumi Yagisawa,¹ Shunsuke Hirooka,² Yuuta Imoto,¹ Kazunobu Matsushita,³ Shigeyuki Kawano,⁴ Tsuneyoshi Kuroiwa^{1*}

In chloroplast division, the plastid-dividing (PD) ring is a main structure of the PD machinery and is a universal structure in the plant kingdom. However, the components and formation of the PD ring have been enigmatic. By proteomic analysis of PD machineries isolated from *Cyanidioschyzon merolae*, we identified the glycosyltransferase protein plastid-dividing ring 1 (PDR1), which constructs the PD ring and is widely conserved from red alga to land plants. Electron microscopy showed that the PDR1 protein forms a ring with carbohydrates at the chloroplast-division site. Fluorometric saccharide ingredient analysis of purified PD ring filaments showed that only glucose was included, and down-regulation of *PDR1* impaired chloroplast division. Thus, the chloroplasts are divided by the PD ring, which is a bundle of PDR1-mediated polyglucan filaments.

For energy, food, and oxygen, almost all organisms depend on photosynthesis by chloroplasts (plastids), which proliferate by division (1–4). During chloroplast division, the FtsZ ring (localized in the stroma) (5–7), the plastid-dividing (PD) ring (in the cytoplasm) (8–10), and the dynamin ring (in the cytoplasm) (11–13) are formed, in that order (2–4, 9, 14). The PD ring is a main structure of the PD ma-

chinery and appears to be a bundle of fine filaments ~5 to 7 nm in diameter (8, 14, 15). Two types of guanosine triphosphatase, a bacterial-derived FtsZ and a eukaryote-specific dynamin, have been characterized as PD machinery-associated proteins (2–4, 11, 12, 15). The unicellular *Cyanidioschyzon merolae* cell contains a single chloroplast and a single mitochondrion (16), and these organelle divisions can be

sequentially synchronized by light/dark cycles (Fig. 1A) (6). In addition, the isolation and analysis of PD machineries have been explored (15), and the complete sequence of the genome has enabled proteomic analysis (16–18).

To detect the components of the PD ring, we digested isolated PD machineries with various proteases but could not completely decompose them. Therefore, we searched for hypothetical components that might constitute the PD ring. Electron-dense deposits (indicating carbohydrates) appeared on the PD ring after staining with periodic acid (PA)-horseradish peroxidase (HRP) (Fig. 1B). The results suggested that the PD ring has a saccharic architecture. To confirm the results, we isolated PD machineries from dividing cells (Fig. 1, A and C to E, and fig. S1). The membrane-free PD machineries formed super-twisted rings, circular rings, and spirals, probably due to the motive force generated

¹Laboratory of Cell Biology, Department of Life Science, College of Science, Research Information Center for Extremophile, Rikkyo University, Toshima, Tokyo 171-8501, Japan. ²Department of Nanobiology, Graduate School of Advanced Integration Science, Chiba University, Matsudo, Chiba 271-8510, Japan. ³Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan. ⁴Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Chiba 277-8562, Japan.

*To whom correspondence should be addressed. E-mail: tsune@rikkyo.ne.jp

Fig. 1. Identification and expression analysis of glycosyltransferase protein PDR1 in *C. merolae* cells. (A) Phase contrast–immunofluorescence images of interphase (left) and dividing (right) cells with the PD machinery (yellow). The PD machinery was immunostained with antibodies against Dnm2 (anti-Dnm2), and the chloroplasts emit red autofluorescence. (B) EM using the PA-HRP method in a cell. The PD machinery was stained in black (bottom). The control image was obtained by the PA-HRP method without avidin/HRP reaction. (Insets) Spectral colors corresponding to the intensity of staining signals of the region around the PD ring (white arrowheads) (see color scales). cp, chloroplast. (C) A scanning electron micrograph of an isolated dividing chloroplast with the PD machinery (yellow arrowhead) from cells. (D and E) Immunofluorescence (D) and immunolectron (E) images of isolated intact circular, supertwisted, and spiral PD machineries. Immunogold particles (indicating Dnm2) localize to the peripheral area of the PD ring. (F) Proteomic analyses of isolated PD machineries (see fig. S2 and table S1). The red arrowhead indicates CMR358C/PDR1. (G) Predicted domain architecture of CMR358C/PDR1. (H) mRNA expression of *PDR1* (red), *Dnm2* (yellow), *FtsZ2-1* (green), and *FtsZ2-2* (blue) throughout the cell cycle, determined with the use of the microarray. The light-dependent gene *Tic22* (gray) is also shown as a control. (I) Protein expression of PDR1, Dnm2, and FtsZ2-1 throughout the cell cycle. (J) Immunoblot analysis with anti-PDR1 antibodies on the fractionated PD machineries with outer membranes as the pellet (P) and stromal components as the supernatant (S). Dnm2, FtsZ2-1, and stAPX (stromal protein) are also shown. Scale bars, 1 μ m [(A) and (D)]; 500 nm [(B) and (C)]; 200 nm (E).

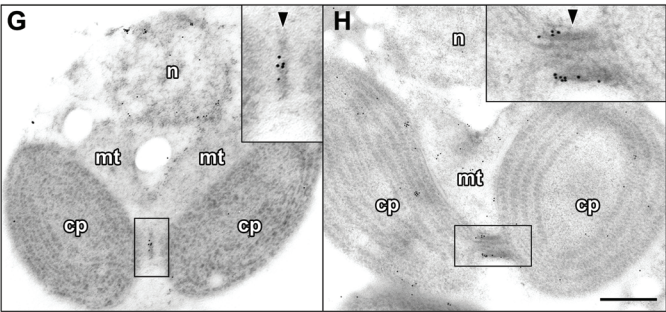
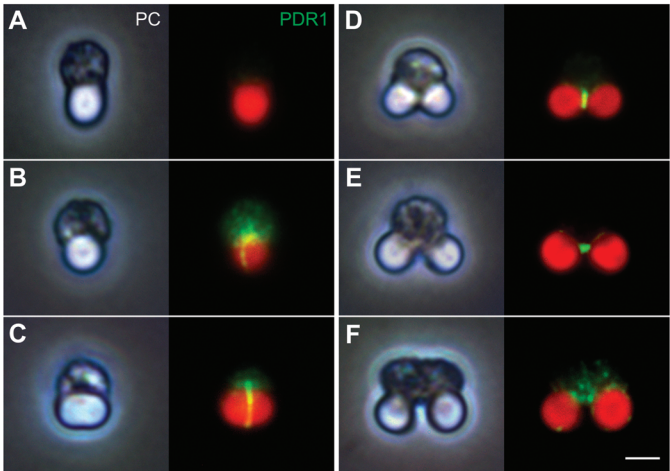
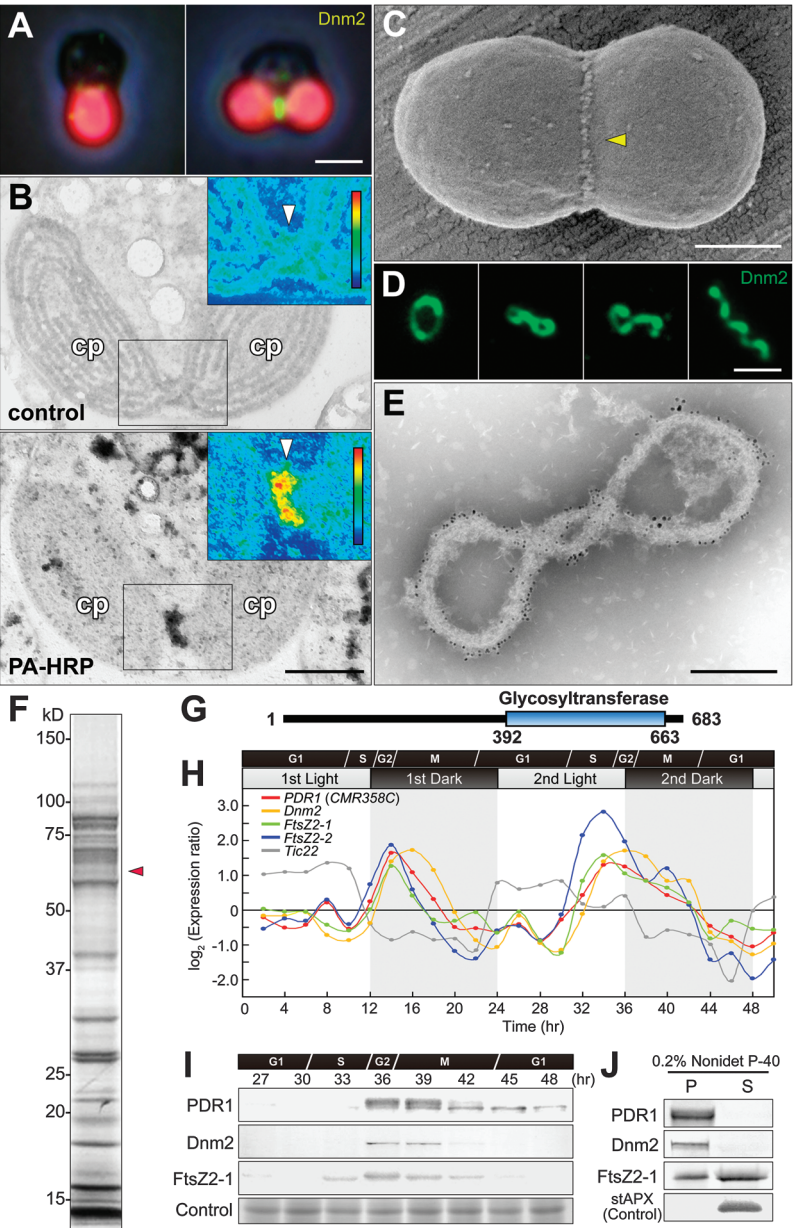


Fig. 2. Immunofluorescence and immuno-EM images of PDR1 in *C. merolae* cells. (A to F) Phase contrast and immunofluorescence images of PDR1 (green) in the cell. More detailed images (immunofluorescence images of PDR1 and Dnm2) are shown in fig. S4. The chloroplasts emit red autofluorescence. (G and H) Immuno-EM micrographs of PDR1 in cells at early (G) and late (H) chloroplast division. (Insets) Magnified images of the region around the PD ring (black arrowheads). n, nucleoid; mt, mitochondria. Scale bars, 1 μ m [(A) to (F)]; 500 nm [(G) and (H)].

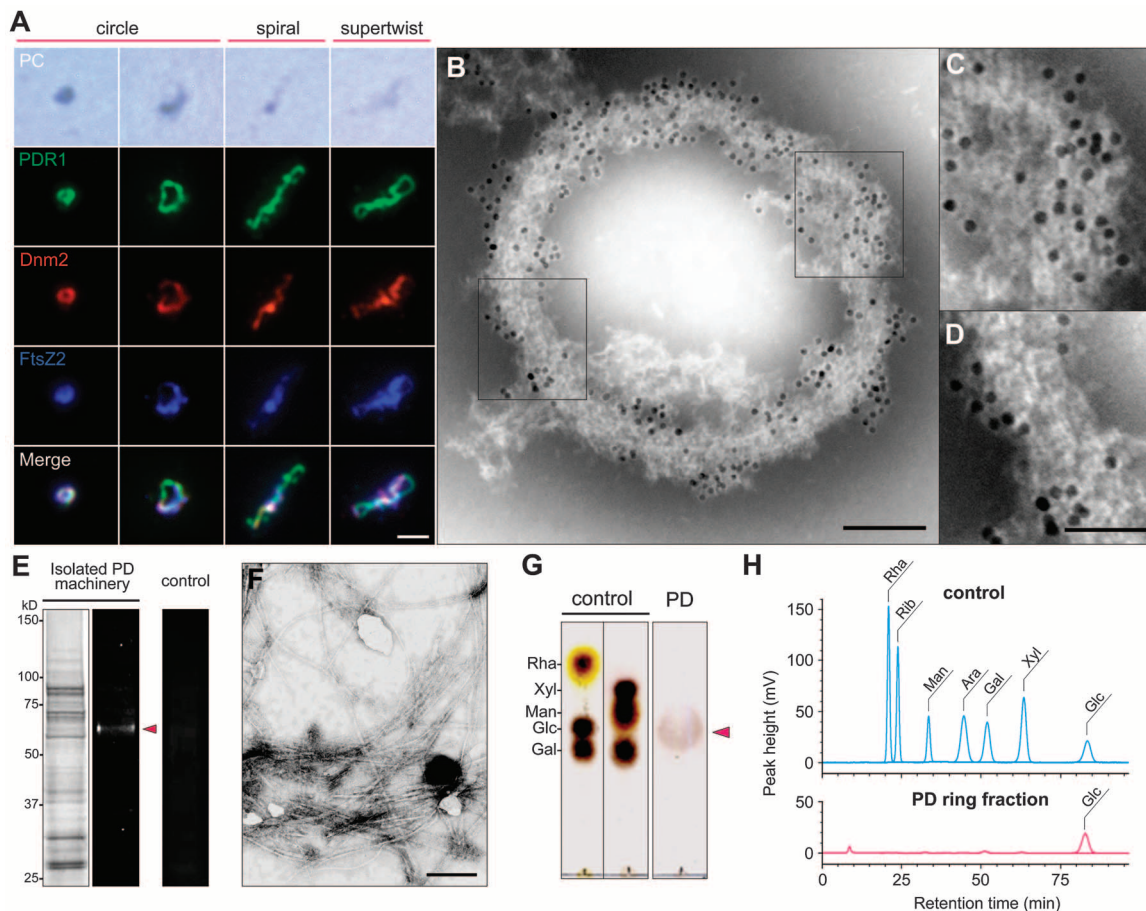
for contraction by dynamin (Fig. 1, D and E) (15, 18). Isolated PD machineries were subjected to proteomic analysis using gradient gel SDS-polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionization-time-of-flight

mass spectrometry (MALDI-TOF MS) (Fig. 1F and fig. S2). We identified a glycogenin-like protein, *CMR358C*, which is part of a subgroup of glycosyl-transferase proteins, and designated it as *plastid-dividing ring 1* (*PDR1*) (Fig. 1F, red arrowhead, fig.

S2, and table S1). Glycogenin is a self-glucosylating protein and is required for the initiation of glycogen synthesis as a priming protein (19). As *PDR1* was not involved in glucan granules purified from *C. merolae* (20), it is thought that *PDR1* is not a

Fig. 3. Localization of

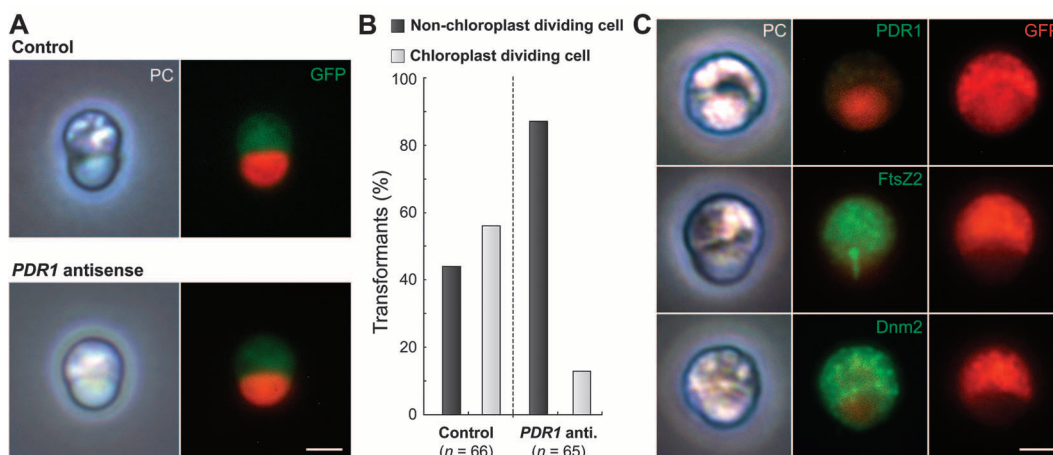
PDR1 in the PD machinery. (A) Phase contrast (PC) and immunofluorescence images of *PDR1*, Dnm2, and FtsZ2-1 in the isolated intact PD machineries. (B) Immuno-EM micrographs of *PDR1* in the PD machinery. Immunogold particles (indicating *PDR1*) localized on the whole of the PD ring. (C and D) Enlarged micrographs of the boxed areas in (B). More immunogold particles appeared in the less condensed F-PDR region (C) than in the solid F-PDR region (D). (E) Fluorometric detection of glycoproteins in the one-dimensional PAGE gel of isolated PD-machinery fraction. After Coomassie brilliant blue staining, the band with the strongest fluorescence signal (red arrowhead) was analyzed by MALDI-TOF MS and detected as *PDR1* (see table S2). The control sample is the insoluble fraction of isolated outer chloroplast membranes after organelle membrane dissolution buffer treatment in interphase cells. (F) Negatively stained electron micrograph of the purified filamentary fraction from isolated PD machineries. (G) TLC-profiles of acid-hydrolysis products of the filamentary fraction. TLC-profile of hydrolyzed filamentary fraction (PD) shows only glucose (red arrowhead). (H) Fluorometric detection of



acid-hydrolysis products of the filamentary fraction in HPLC using arginine. Post-column fluorometric detection of reducing sugars in HPLC through the use of arginine is a highly sensitive determination of reducing sugars. The HPLC profile also shows that the filamentary fraction (bottom) involves only glucose. Scale bars, 1 μ m (A); 100 nm (B); 50 nm [(C) and (D)]; 500 nm (F).

Fig. 4. Down-regulation of *PDR1*.

(A) Phase contrast and fluorescence images of control and antisense-*PDR1* cells. GFP, green fluorescent protein. (B) In cells with transient DNA introduction and expression of *sGFP* (control), chloroplast division occurred normally. In cells in which *PDR1* expression was down-regulated by antisense suppression, the frequency of chloroplast division is reduced ($p < 0.001$, by Fisher's exact test). Data are the total number of transformants examined (n) in more than five replicates. (C) Phase contrast and immunofluorescence images of *PDR1*, Dnm2, and FtsZ2-1 of cells treated with antisense-*PDR1*. Scale bars, 1 μ m [(A) and (C)].



storage glucan synthase. A characteristic feature of PDR1 is a putative glycosyltransferase domain in the C-terminal region (Fig. 1G). Homologs of *PDR1* are encoded by the genomes of red alga and land plants (fig. S3). The level of transcription of *PDR1* increased during the S to M phase, and the expression profile of *PDR1* coincided with those of other known PD machinery-associated proteins [for instance, dynamin (Dnm2) and FtsZ (FtsZ2)] (Fig. 1H). We then used antibodies against PDR1 to analyze protein expression during the cell cycle (Fig. 1I). Although the expression of PDR1 was detected during chloroplast division, the immunoblot band corresponding to PDR1 was shifted slightly downward, becoming a sharp band, and was subsequently decreased (Fig. 1I). This led us to hypothesize that PDR1 might be glycosylated like glycogenin in the S to G₂ phase and then slowly decomposes. Immunoblot analyses of isolated PD machineries showed that almost all PDR1 and dynamin (and some FtsZ) associated with the PD machineries in the outer-chloroplast membrane fraction and that PDR1 and dynamin were most concentrated in the isolated PD-machineries fraction (Fig. 1J and fig. S1). Thus, PDR1 was determined to be a PD-machinery-specific protein.

Immunofluorescence microscopy demonstrated that PDR1 appeared earlier than dynamin in the cytoplasm and formed a ring from the beginning to the end of chloroplast division (Fig. 2, A to E, and fig. S4). When chloroplast division was complete, the immunofluorescence signal of PDR1 was dispersed in the cytoplasm (Fig. 2F). Furthermore, immuno-electron microscopy (EM) of PDR1 showed that immunogold particles (indicating PDR1) were colocalized on the PD ring throughout chloroplast division (Fig. 2, G and H, and fig. S5). A series of dynamic molecular movements of PDR1 in the cell during chloroplast division coincided with those of the PD ring. The results suggested that the PDR1 is a component of the PD ring.

To reveal the relation between PDR1 and PD ring filaments (F-PDRs), we isolated intact PD machineries. Immunofluorescence microscopy showed that PDR1 was localized uniformly on each of the PD machineries throughout chloroplast division, whereas dynamin and FtsZ were unequally localized on the machineries, suggesting that PDR1 is a structural component of the PD machineries (Fig. 3A). Immuno-EM showed immunogold particles (indicating PDR1) localized all over each PD machinery derived from various stages of chloroplast division (Fig. 3B and fig. S6). Many more immunogold particles appeared in the less condensed F-PDR region (Fig. 3C) than in the solid F-PDR region (Fig. 3D), suggesting that PDR1 proteins are associated with the whole of the PD ring, from the inside to the outside. In a magnified image of the less condensed PD-ring region, immunogold particles (indicating PDR1) could be recognized along the edge of each of the F-PDRs (fig. S7). In contrast, immunogold particles (indicating

dynamin) were localized on peripheral areas of the PD ring (fig. S7), as shown previously (Fig. 1E) (15, 18).

To examine whether PDR1 binds to saccharides, we performed a glycoprotein-detection analysis (21). A strong fluorometric signal was detected in the isolated PD-machineries fraction, whereas no signal was detected in a control sample derived from interphase cells. The signal was confirmed to derive from PDR1 by MALDI-TOF MS (Fig. 3E and table S2). This result supports the hypothesis from the immunoblot experiment that PDR1 is glycosylated only during the chloroplast-division phase (Fig. 1I). Moreover, after removal of proteins from isolated PD machineries by protease and ionic treatments (21), many fine filaments of 5 to 7 nm in diameter remained (Fig. 3F). These filaments were hydrolyzed and analyzed by carbohydrate analysis with the use of thin-layer chromatography (TLC) (Fig. 3G) or high-performance liquid chromatograph (HPLC) with postlabel fluorometric detection (Fig. 3H and table S3) (21). The results showed that the filamentary fraction contained only glucose (Fig. 3G, red arrowhead, and Fig. 3H, bottom). In addition, the filamentary fraction was not digested by some glucosidases, but it dissolved in dimethyl sulfoxide (table S4), as does α -glucan (22). These results suggest that the F-PDRs are composed of PDR1-associated polyglucan chains.

To further support the finding that PDR1 is involved in chloroplast division, we examined down-regulation of *PDR1* by antisense suppression (Fig. 4). Transient DNA introduction and expression of antisense *PDR1* in cells were performed by a polyethylene glycol transformation protocol (18). At 24 hours after the start of cultivation, we determined whether or not chloroplast division occurred. The results showed that antisense *PDR1* significantly decreased the frequency of chloroplast division compared with control cells ($p < 0.001$; Fisher's exact test) (Fig. 4, A and B). Immunofluorescence microscopy showed that, in the antisense *PDR1* cells, PDR1 and dynamin rings were not formed, but the FtsZ ring was formed at the chloroplast-division site (Fig. 4C). Dynamin signals appeared in the cytosol (Fig. 4C). The results suggest that during chloroplast division, PDR1 is required for the formation of the PD ring and the organization of dynamin at the division site after formation of the FtsZ ring.

Various proteins have been found in the PD machineries by proteomic analysis with MALDI-TOF MS (fig. S2 and table S1) (18). The present results suggest that the PD ring is composed of PDR1-associated polyglucan chains and proteins. Priming glycosyltransferases, such as glycogenin, have a fundamental property of polysaccharide chain synthesis (23). Glycosylated-glycogenin elongates the glucan chain by the sequential addition of glucose residues from uridine diphosphate-glucose to synthesize glycogen (19, 23). Moreover, immunogold particles indicated that PDR1 is attached to the F-PDRs (Fig. 3, B to D, and figs. S6

and S7); therefore, we hypothesize that PDR1 constructs polyglucan filaments as a priming glycosyltransferase by a similar synthetic mechanism to that of glycogenin. Serial analysis of PDR1 showed the formation of the PD machinery (fig. S8). After the formation of the FtsZ ring, PDR1 proteins are recruited at the chloroplast-division site on the outer membrane and might be responsible for synthesizing F-PDRs, which probably consist of α -glucan (table S4). As the transcriptions of glycogen/starch metabolism genes were not increased during chloroplast division (fig. S9), synthesis of F-PDR might be performed by PDR1 itself or by an unidentified unique glucan synthase. Thus, the PD ring is thought to be formed as a filamentary bundle that includes PDR1 (fig. S8). After the formation of the PD ring, dynamin is recruited from the cytoplasm to the surface of the PD ring and binds between the outermost F-PDRs to generate the motive force for contraction (fig. S8) (11, 15). Homologs of *PDR1* are widely conserved in the plant kingdom (fig. S3); therefore, our findings concerning the PD ring will increase our understanding of the common chloroplast proliferation mechanism. As glycosyltransferase domains have been found in bacteria, it is thought that host eukaryotes acquired PDR1 from an engulfed bacterial glycosyltransferase domain with modifications and additional regions in order to facilitate chloroplast division.

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Conserved Fungal LysM Effector Ecp6 Prevents Chitin-Triggered Immunity in Plants

Ronnie de Jonge,¹ H. Peter van Esse,¹ Anja Kombrink,¹ Tomonori Shinya,³ Yoshitake Desaki,³ Ralph Bours,⁴ Sander van der Krol,⁴ Naoto Shibuya,³ Matthieu H. A. J. Joosten,¹ Bart P. H. J. Thomma^{1,2*}

Multicellular organisms activate immunity upon recognition of pathogen-associated molecular patterns (PAMPs). Chitin is the major component of fungal cell walls, and chitin oligosaccharides act as PAMPs in plant and mammalian cells. Microbial pathogens deliver effector proteins to suppress PAMP-triggered host immunity and to establish infection. Here, we show that the LysM domain-containing effector protein Ecp6 of the fungal plant pathogen *Cladosporium fulvum* mediates virulence through perturbation of chitin-triggered host immunity. During infection, Ecp6 sequesters chitin oligosaccharides that are released from the cell walls of invading hyphae to prevent elicitation of host immunity. This may represent a common strategy of host immune suppression by fungal pathogens, because LysM effectors are widely conserved in the fungal kingdom.

Multicellular organisms activate immune responses upon recognition of microbe-derived nonself components. These responses are mediated by pattern recognition receptors (PRRs), cell surface receptors that recognize invariant structures, usually originating from microbial surfaces that are essential for microbial survival and not present in the host. These microbial structures are known as pathogen-associated molecular patterns (PAMPs) (1–4). Well-known PAMPs include bacterial lipopolysaccharides, peptidoglycans, flagellin, and fungal cell wall-derived glucans and mannans (4–6). Also chitin, an unbranched β -1,4-linked *N*-acetyl-glucosamine (GlcNAc) homopolymer that is the major structural component of fungal cell walls, acts as a PAMP in many organisms (6–8). In the plant species rice and *Arabidopsis*, single PRRs were shown to be required for the activation of host immunity upon chitin perception (9–12). Mutants in these receptors are compromised in their response to chitin and are impaired in their defense against chitin-containing fungal pathogens, which indicates that perception

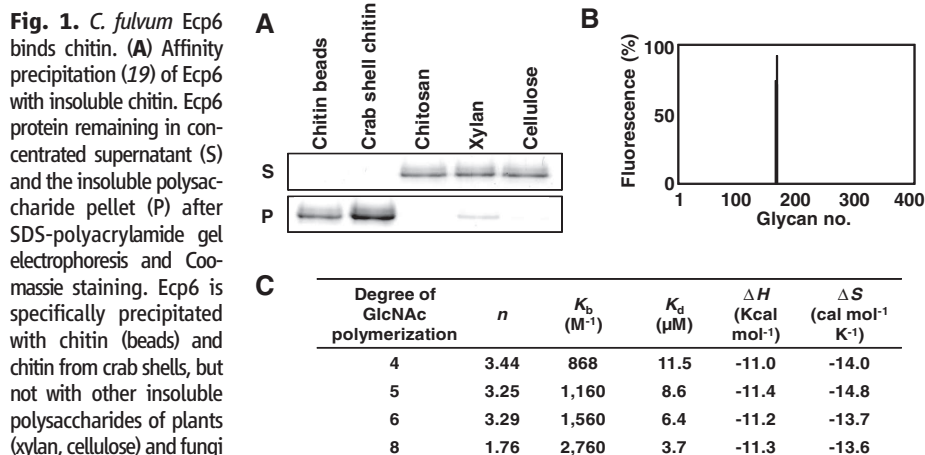
of chitin fragments plays a pivotal role in resistance of plants to fungal pathogens. Both chitin receptors of rice and *Arabidopsis* were shown to contain extracellular LysM domains that generally occur in glycan-binding proteins (9–13).

Cladosporium fulvum is a fungal pathogen that causes leaf mold of tomato (*Solanum lycopersicum*) (14). During colonization of the intercellular spaces of the leaves, the fungus secretes

effector proteins to establish disease, one of which, Avr4, is a chitin-binding lectin that protects fungal cell walls against hydrolysis by plant chitinases (15, 16). Recently, the in planta abundantly secreted *C. fulvum* LysM domain-containing effector Ecp6 was identified and shown to be required for full virulence (17, 18).

We first examined the affinity of Ecp6 for insoluble polysaccharides because the presence of three LysM domains in Ecp6 suggested that it has glycan-binding activity. Ecp6 showed specific affinity for chitin, as it coprecipitated with insoluble chitin (chitin beads and crab shell chitin), but not with chitosan (i.e., deacetylated chitin) or the plant cell wall polysaccharides xylan and cellulose (19) (Fig. 1A). To further examine Ecp6 substrate specificity, a glycan array was used to test the affinity of Ecp6 for more than 400 different glycan substrates (20). Ecp6 only bound to the three chitin oligosaccharides present on the array, (GlcNAc)₃, (GlcNAc)₅, and (GlcNAc)₆, but not to any other glycan, including the N-linked glycan chitobiose (Fig. 1B and table S1). Therefore, we conclude that Ecp6 is a highly specific chitin-binding LysM effector.

The affinity of Ecp6 for soluble chitin oligosaccharides was determined by isothermal titration calorimetry (ITC). The binding curves for chitin tetra-, penta- and hexamer oligosaccharides [(GlcNAc)₄, (GlcNAc)₅, and (GlcNAc)₆, respectively] obeyed a "one binding site" model, revealing three binding sites for these oligosaccharides per Ecp6 molecule, which matches with the three



¹Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, Netherlands.

²Centre for Biosystems Genomics, Post Office Box 98, 6700 AB Wageningen, Netherlands. ³Department of Life Sciences, Faculty of Agriculture, Meiji University, Kawasaki, Kanagawa 214–8571, Japan. ⁴Laboratory of Plant Physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, Netherlands.

*To whom correspondence should be addressed. E-mail: bart.thomma@wur.nl

LysM domains in Ecp6 (fig. S1, A to C). The (GlcNAc)₈ binding curve deviated from this model, which suggested that the size of this octamer allows it to interact with multiple LysM domains simultaneously (fig. S1D). The dissociation constant (*K*_d) for the various GlcNAc oligosaccharides was similar and decreased from 11.5 to 3.7 μM between (GlcNAc)₄ and (GlcNAc)₈ (Fig. 1C), which showed that Ecp6 had high affinity for chitin oligosaccharides of various lengths. It was previously determined that the invertebrate (CBM14) chitin-binding domain of Avr4 exclusively interacts with (GlcNAc)₃ repeats and that the Avr4 *K*_d decreased from 1.3 mM μM to 6.3 μM between (GlcNAc)₄ and (GlcNAc)₆ (21). This shows that, in contrast to Ecp6, Avr4 has low affinity for short-chain chitin oligosaccharides.

Avr4 fully protects fungal cell walls against hydrolysis by plant chitinases at a concentration of 10 μM (15, 16). Despite its chitin-binding activity, however, 10 μM or 100 μM Ecp6 failed to protect the fungus *Trichoderma viride* against hydrolysis by crude extracts of tomato leaves containing intracellular basic chitinases (Fig. 2A and fig. S2). We conclude that Ecp6 effector function did not involve prevention of the hydrolysis of fungal cell walls by plant chitinases.

Apart from hydrolyzing fungal cells, host chitinases cause the release of chitin oligosaccharide PAMPs from the cell walls of the invading fungus (6, 22). Suspension-cultured plant cells react with medium alkalization to treatment with nanomolar concentrations of chitin oligosaccharides (8). We speculated that LysM effectors might affect chitin perception by the host (17, 18) and tested the effect of Ecp6 treatment on PAMP-triggered immunity by measuring chitin-induced pH shifts in tomato and tobacco cell suspensions. Treatment of the cells with nanomolar chitin oligosaccharide [(GlcNAc)₆] concentrations resulted in medium alkalization, whereas addition of equimolar amounts of Ecp6 indeed attenuated this response (Fig. 2B). The pH shift attenuation occurred in a dose-dependent manner and, similarly, occurred with various chitin oligosaccharides of different lengths and in both tomato and tobacco suspensions (Fig. 2B and fig. S3A). In contrast, even a 10-fold molar excess of Avr4 did not affect the chitin-induced pH shift (Fig. 2B and fig. S3B). Similarly, a 10-fold excess of the *C. fulvum* effectors Avr9, Ecp1, and Ecp4, which, like Ecp6, are small cysteine-rich proteins that are abundantly secreted in the apoplast during colonization of the host plant but that do not bind chitin (15) (fig. S3C), did not affect the chitin-induced pH shift (fig. S3B). These data show that only Ecp6 is able to suppress chitin-triggered immunity. The control oligosaccharides laminarihexaose (β-1,3-glucan), D-cellohexaose (β-1,4-glucan), chitosan hexamer (GlcN)₆, and chitosan did not induce a pH shift in tomato cell suspensions (fig. S3D). As expected, Ecp6 did not inhibit alkalization induced by the bacterial PAMP flg22, the epitope of bacterial flagellin, which suggested that sup-

Fig. 2. Ecp6 cannot protect fungal hyphae from hydrolysis by tomato chitinases but inhibits chitin-induced medium alkalization of tomato cell suspensions. **(A)** Micrographs of *Trichoderma viride* taken 24 hours after the addition of water (w), crude extract of tomato leaves containing intracellular, basic chitinases (ChiB), pretreatment with 10 μM Ecp6 followed by addition of tomato extract (Ecp6 & ChiB), and pretreatment with 10 μM Avr4 followed by addition of tomato extract (Avr4 & ChiB). Scale bars, 50 μm. The figure is representative of three independent experiments. **(B)** Medium alkalization of tomato cell suspensions induced by chitin oligosaccharides is inhibited by Ecp6. The Δ*pH*_{max} determined after treatment of tomato cell suspensions with mixtures of chitin oligosaccharides (GlcNAc)₆ and Ecp6, after normalization to the response upon treatment with 10 nM (GlcNAc)₆ only, is indicated (19). Bars represent means ± standard error of at least three replicate experiments. Statistically significant differences when compared to treatment with 10 nM (GlcNAc)₆ were determined using the Dunnett test (two-sided; **P* < 0.05).

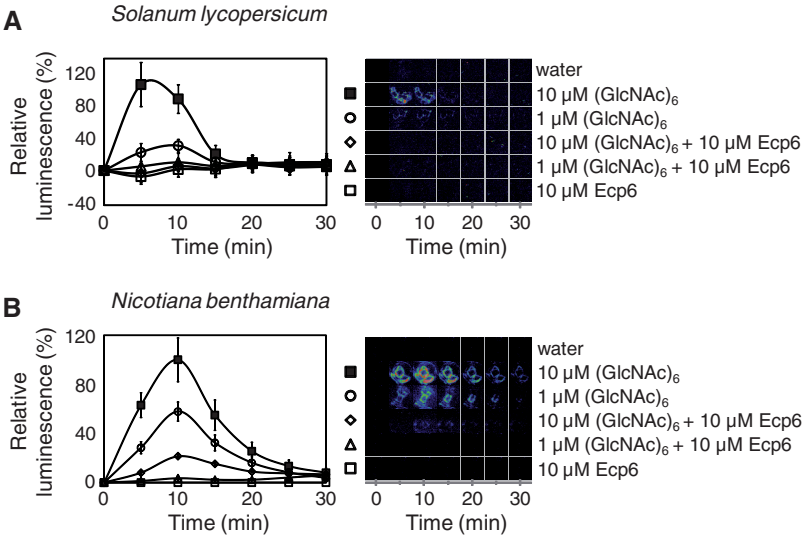
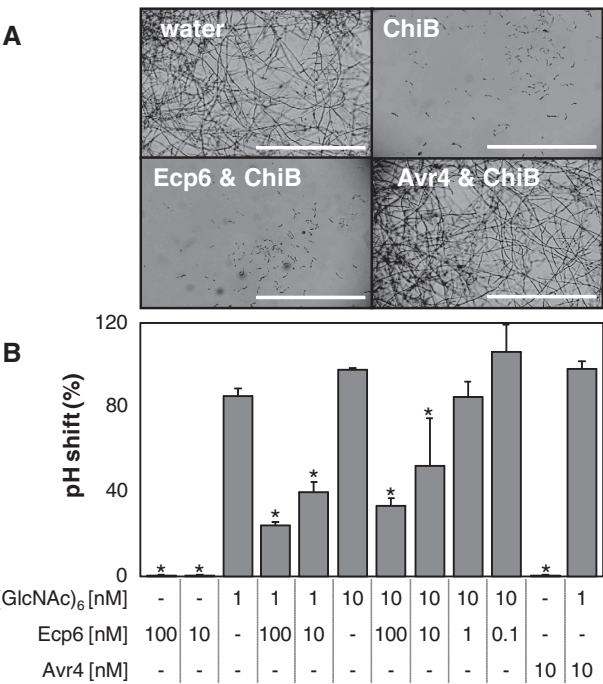


Fig. 3. The chitin-induced oxidative burst in **(A)** tomato, *Solanum lycopersicum*, and **(B)** tobacco, *N. benthamiana*, leaf discs is inhibited by Ecp6. Production of ROS was determined using luminol-dependent chemiluminescence (19). (Left) Integrated images of 5-min exposures were analyzed with ImageJ, and the relative luminescence was calculated by normalization to water-treated leaf discs and plotted. (Right) Representative image sequence of a single experiment showing the ROS-dependent luminescence over time in leaf discs treated with one of the following: water, 10 μM (GlcNAc)₆ (■), 1 μM (GlcNAc)₆ (◇), a mixture of 10 μM (GlcNAc)₆ and 10 μM Ecp6 (◊), a mixture of 1 μM (GlcNAc)₆ and 10 μM Ecp6 (△), and 10 μM Ecp6 (□). The figure is representative of three independent experiments.

pression of chitin-triggered immunity by Ecp6 occurs through specific binding of chitin oligosaccharide PAMPs (fig. S3E).

These findings were further substantiated in experiments to assess whether, besides cell suspensions, Ecp6 also perturbs chitin-induced host

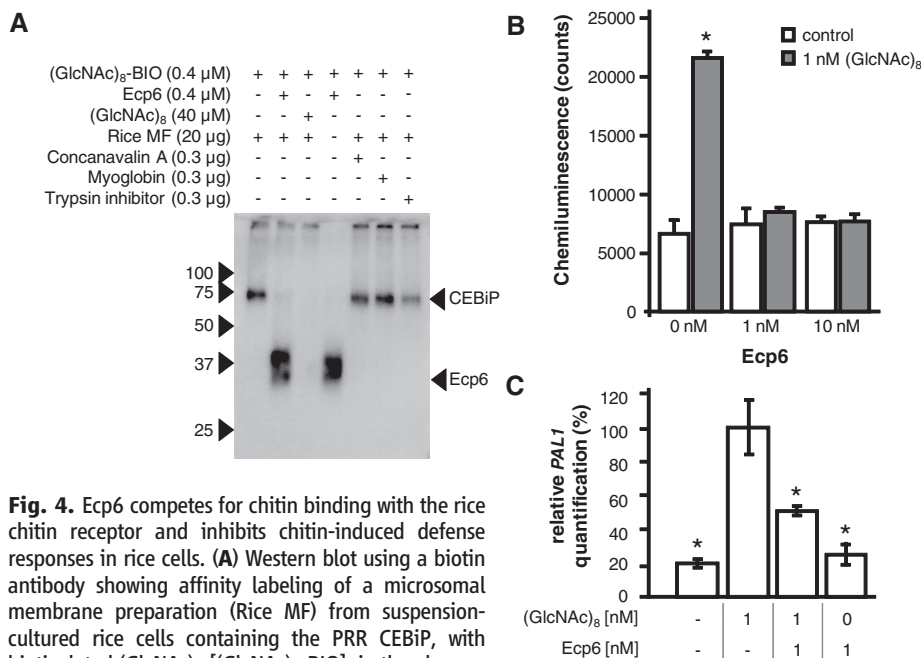


Fig. 4. Ecp6 competes for chitin binding with the rice chitin receptor and inhibits chitin-induced defense responses in rice cells. **(A)** Western blot using a biotin antibody showing affinity labeling of a microsomal membrane preparation (Rice MF) from suspension-cultured rice cells containing the PRR CEBiP, with biotinylated (GlcNAc)₈ [(GlcNAc)₈-BIO], in the absence or presence of Ecp6, nonbiotinylated (GlcNAc)₈, and the control proteins concanavalin A, myoglobin, and trypsin inhibitor. The experiment was performed twice with similar results. **(B)** Ecp6 inhibits the chitin-induced oxidative burst in rice suspension cells. Production of ROS 20 min after induction with 1 nM (GlcNAc)₈ was determined as described previously (9) in the absence or presence of Ecp6 (1 and 10 nM). The experiment was performed twice with similar results. **(C)** Ecp6 inhibits chitin-induced *PAL1* gene expression in rice suspension cells. The bars display the relative transcript level of the chitin-responsive gene *PAL1* normalized to the constitutively expressed ubiquitin gene, and the relative transcript level of the suspension cells treated with 1 μM (GlcNAc)₈ was set at 100%. The mean with standard error of two replicate experiments is shown, and asterisks indicate significant differences ($P < 0.05$) when compared with the 1 μM (GlcNAc)₈ treatment.

immunity in tomato and tobacco leaves. Treatment of leaf discs with (GlcNAc)₆ resulted in the production of reactive oxygen species (ROS), whereas this response was abolished in the presence of Ecp6 (Fig. 3). Furthermore, inhibition of the induction of chitin-responsive genes in the presence of Ecp6 was recorded in tomato leaves (fig. S4). Similar to the alkalization response in cell suspensions, Avr4 and Avr9 did not affect the chitin-induced ROS production, and Ecp6 could not inhibit the flg22-induced ROS burst (fig. S5).

Finally, we tested whether Ecp6 is able to compete directly for chitin binding with a plant PRR. It has previously been demonstrated that the rice PRR CEBiP directly binds chitin oligosaccharides (9). As chitin binding was shown to this receptor, we performed competition assays in which a microsomal membrane preparation from suspension-cultured rice cells containing CEBiP was treated with biotinylated (GlcNAc)₈ in the presence or absence of Ecp6 (23). Although incubation of microsomal membranes with 0.4 μM biotinylated (GlcNAc)₈ resulted in labeling of the CEBiP receptor, incubation in the presence of a 100-fold molar excess of nonbiotinylated (GlcNAc)₈ prevented receptor labeling (Fig. 4). Incubation of microsomal membranes with biotinylated (GlcNAc)₈ in the presence of an equimolar amount of Ecp6 almost completely prevented receptor labeling

while a signal at the height of Ecp6 was observed, which demonstrated that Ecp6 directly competes for chitin binding with the CEBiP chitin receptor (Fig. 4).

In conclusion, our data show that the abundantly secreted *C. fulvum* LysM effector Ecp6 is a chitin-binding lectin that inhibits activation of chitin-triggered host immunity. Thus, at present, two chitin-binding *C. fulvum* effectors have been identified; Avr4, which carries an invertebrate chitin-binding domain with high affinity for long-chain chitin oligosaccharides and which protects fungal hyphae against lysis by plant chitinases, and Ecp6, which carries LysM domains with high affinity for various short-chain chitin oligosaccharides and which prevents activation of chitin PAMP-triggered immunity. These distinct activities of both effectors corroborate the finding that Avr4 protects hyphae against hydrolysis by basic, vacuolar, endochitinases that are released by the host upon cellular collapse (15, 16), but not necessarily against exochitinases that are present in the apoplast and that are able to release short-chain chitin oligosaccharides from the fungal cell wall. Besides differing from Avr4, the scavenger function of Ecp6 also differs from the role of the effector Avr2, which is secreted by *C. fulvum* to inhibit extracellular tomato cysteine proteases that are required for host basal defense (24–26).

Ecp6 orthologs are present in many fungi, often occurring in multigene families (18). This suggests that scavenging of chitin oligosaccharides to avoid perception by other organisms may be an important survival strategy of fungi.

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Linear Arrays of Nuclear Envelope Proteins Harness Retrograde Actin Flow for Nuclear Movement

G. W. Gant Luxton,^{1*} Edgar R. Gomes,^{1,2,3*} Eric S. Folker,¹ Erin Vintinner,¹ Gregg G. Gundersen^{1†}

Nuclei move to specific locations to polarize migrating and differentiating cells. Many nuclear movements are microtubule-dependent. However, nuclear movement to reorient the centrosome in migrating fibroblasts occurs through an unknown actin-dependent mechanism. We found that linear arrays of outer (nesprin2G) and inner (SUN2) nuclear membrane proteins assembled on and moved with retrogradely moving dorsal actin cables during nuclear movement in polarizing fibroblasts. Inhibition of nesprin2G, SUN2, or actin prevented nuclear movement and centrosome reorientation. The coupling of actin cables to the nuclear membrane for nuclear movement via specific membrane proteins indicates that, like plasma membrane integrins, nuclear membrane proteins assemble into actin-dependent arrays for force transduction.

Directed nuclear movements are important for establishing cellular polarity during cell migration, development, and homeostasis and ensuring the equal distribution of genetic material during cell division (1–3). Most nuclear movements are microtubule-mediated; however, growing numbers of actin-dependent nuclear movements have been recognized (1, 2, 4–10).

Mechanisms for actin-dependent nuclear movement are unclear.

In NIH3T3 fibroblasts polarizing for migration into *in vitro* wounds, an actin-dependent nuclear movement is triggered by serum or the serum factor lysophosphatidic acid (LPA), and this reorients the centrosome toward the leading edge (5). Nuclear movement and actin retrograde

flow occur at the same rate, but how actin is coupled to the nucleus is unknown. We explored the possible involvement of the LINC (linker of nucleoskeleton and cytoskeleton) complex, which spans the inner and outer nuclear membranes (INM and ONM, respectively). LINC complexes consist of ONM nesprin and INM SUN proteins and have been implicated in microtubule-dependent, but not actin-dependent, nuclear movements (2, 11–13). Nevertheless, the largest splice forms of two mammalian nesprins, nesprin1 and nesprin2, contain cytoplasmically oriented paired actin-binding calponin homology (CH) domains.

To test whether nesprins were involved in nuclear movement, we initially expressed dominant negative constructs [red fluorescent protein–Klarsicht/ANC-1/Syne homology (RFP-SR-KASH) and RFP-KASH] of the LINC complex in wound-edge NIH3T3 fibroblasts and then stimulated

¹Department of Pathology and Cell Biology, Columbia University, New York, NY 10032, USA. ²UMR 5 787 INSERM, Université Pierre et Marie Curie Paris VI, 75634 Paris, France. ³Groupe Hospitalier Pitié-Salpêtrière, Institut de Myologie, 75013 Paris, France.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: ggg1@columbia.edu

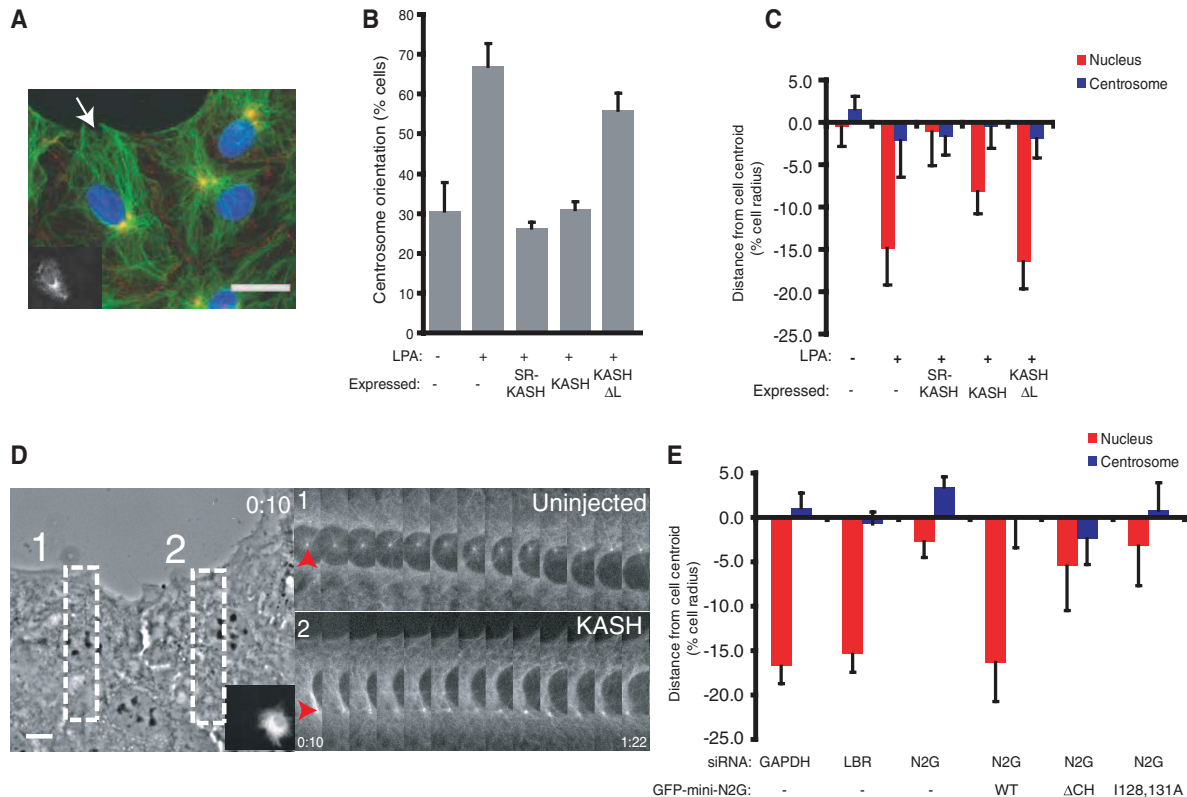


Fig. 1. Nuclear movement requires nesprin2G. Wound edge is toward the top of all images. **(A)** Representative wide-field epifluorescence image of centrosome orientation in RFP-KASH-expressing cells (cell expressing RFP-KASH is shown in inset and by arrow). Cells were stained as follows: DNA (blue), centrosomes (anti-pericentrin, yellow), microtubules (green), and cell-cell contacts (anti- β -catenin, red). **(B)** Centrosome orientation in cells expressing the indicated constructs. Random reorientation is ~33% (27). **(C)** Average centrosome and nucleus positions along the axis perpendicular to the wound from cells described in (B). The cell center is defined as "0." Positive

values are toward leading edge; negative values, away. **(D)** Nuclear movement in RFP-KASH-expressing (inset), GFP- α -tubulin NIH3T3 fibroblasts. (Left) Phase-contrast image from the start of movie S1. Boxes indicate regions used for the GFP- α -tubulin fluorescence kymographs on the right. Arrowheads indicate centrosomes. Time is in hour:min. **(E)** Average centrosome and nucleus positions from siRNA-treated cells expressing the indicated GFP-tagged constructs. LBR is lamin B receptor; WT, wild type; N2G, nesprin2G. Experiments were repeated ≥ 3 times [$N > 30$ for (B) and (C); $N > 33$ for (E)]. Error bars indicate SEM. Scale bars in (A), 15 μ m; (D), 10 μ m.

Fig. 2. Coupled movement of dorsal actin cables and the nucleus. The wound edge is toward the top of all images. (A) Representative wide-field epifluorescence images of nuclei in cells fixed after LPA-treatment. Time is in min. Cells were stained as follows: DNA [4',6'-diamidino-2-phenylindole (DAPI)] and F-actin (rhodamine-phalloidin). (B) Number of dorsal cables spanning the entire nucleus from cells treated as in (A). Experiments were repeated three times with $N > 151$. (C) Fluorescence kymograph of a Lifeact-mCherry-expressing cell from movies S4 and S5. Fluorescence images were acquired from dorsal and ventral planes of the cell, pseudocolored, and merged. Nuclear position, dashed white circle. Arrows indicate dorsal (red) and ventral (green) cables. Images taken every 5 min. (D) Fluorescence kymographs of Lifeact-mCherry in GAPDH- or nesprin2G-depleted cells. Nuclear position, red dashed circle. Images are every 10 min. Arrows indicate moving dorsal actin cables. (E) LPA-stimulated dorsal cable and nucleus velocities in cells treated as in (D). Data are from five independent experiments [$N = 36$ (GAPDH) and 27 (nesprin2G)]. Error bars indicate SEM. Scale bars in (A) and (C), 5 μm ; (D), 10 μm .

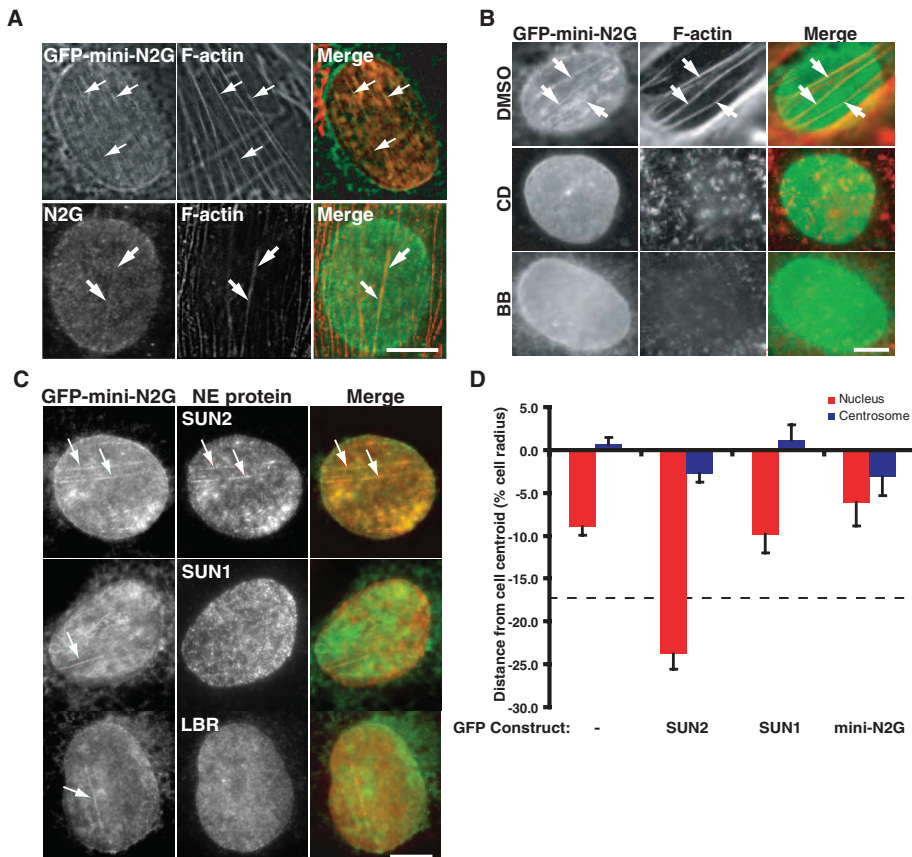
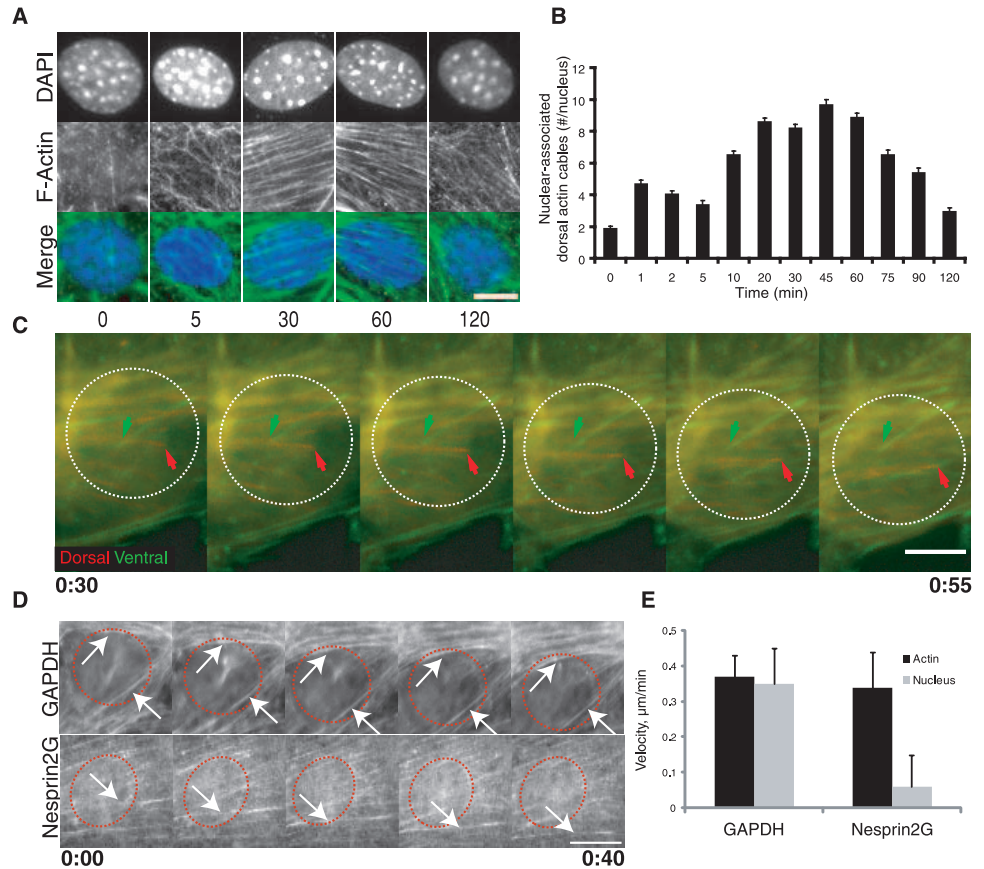


Fig. 3. Nesprin2G and SUN2 form TAN lines. All images are of the dorsal nuclear surface. (A) (Top) Fluorescence images of a nucleus from a nesprin2G-depleted cell expressing GFP-mini-N2G and stained with rhodamine-phalloidin (F-actin). (Bottom) Fluorescent images of a nucleus in a cell stained with nesprin2G antibody (N2G) and rhodamine-phalloidin (F-actin). Arrows, colocalized N2G and dorsal actin cables. Wound edges are toward the upper right (top) and right (bottom). (B) Fluorescence images of nuclei in nesprin2G-depleted cells expressing GFP-mini-N2G. Cells were stained with GFP antibody (GFP-mini-N2G) and rhodamine-phalloidin (F-actin). Cells were treated with dimethyl sulfoxide (DMSO), 50 μM blebbistatin (BB), or 0.5 μM cytochalasin D (CD) for 1 hour before and during LPA treatment. The wound edge is toward the top left. (C) Fluorescence images of nuclei in nesprin2G-depleted cells. Staining for SUN1, SUN2, LBR, and GFP (GFP-mini-N2G) antibodies. Arrows show N2G colocalizing with SUN2 but not SUN1 or LBR. The wound edge is toward the top. (D) Average centrosome and nucleus positions from SUN2-depleted cells expressing the indicated GFP-tagged constructs. Dashed line represents average nuclear position in nondepleted cells. Experiments were repeated ≥ 3 times with $N > 201$. Error bars, SEM. Scale bars in (A) to (C), 5 μm .

nuclear movement with LPA (14). Expression of these constructs, known to disrupt LINC complexes and displace nesprins from the nuclear envelope (2, 3, 15), inhibited centrosome orientation and rearward nuclear positioning, whereas a control construct (RFP-KASH Δ L) lacking the luminal SUN-binding domain had no effect (Fig. 1, A to C, and fig. S1). Live cell imaging showed that RFP-KASH blocked nuclear movement (Fig. 1D and movie S1). Thus, nesprins and the LINC complex are involved in centrosome orientation and nuclear movement. We cannot exclude the possibility that nesprins function in centrosome positioning, because nuclear movement is needed to observe centrosome centration defects (5, 16).

Expression analysis and immunoblotting showed that NIH3T3 fibroblasts express only one of the actin-binding, giant nesprin isoforms, nesprin2G (fig. S2, A to C). Depletion of nesprin2G with small interfering RNA (siRNA) blocked centrosome orientation because of defective rearward nuclear movement, whereas control siRNAs had no effect (Fig. 1E, figs. S2 to S4, and movies S2 and S3). These effects of nesprin2G depletion were not due to gross alterations in the nuclear envelope because the levels and localization of five other nuclear envelope proteins were not greatly altered (fig. S4).

The centrosome and nuclear defects in nesprin2G-depleted cells were rescued by expression of green fluorescent protein (GFP)-mini-N2G, which contains the N-terminal CH domains and a

C-terminal region containing spectrin repeats and the KASH domain of nesprin2G (Fig. 1E and figs. S5 and S6). GFP-mini-N2G lacking the CH domains (Δ CH) or mutated to reduce F-actin binding [Ile¹²⁸→Ala¹²⁸ and Ile¹³¹→Ala¹³¹ (abbreviated as I128, 131A in Fig. 1)] failed to rescue the polarity defects in nesprin2G-depleted cells (Fig. 1E and figs. S5 and S6). Thus, nesprin2G and its actin-binding CH domains are necessary for nuclear movement.

We next asked whether moving nuclei associated with actin filaments. LPA stimulates actin filament formation in serum-starved cells (5, 17–19), and we found that an irregular actin meshwork formed near the nucleus at early times after LPA stimulation (Fig. 2A). This meshwork rearranged by the time nuclear movement began (~30 min LPA stimulation) into distinct actin cables on the dorsal and ventral surfaces of the cell (Fig. 2, A and B, and fig. S7). The dorsal cables were usually parallel to the leading edge, and resembled transverse actin arcs previously described in migrating cells (20, 21). The ventral cables were typically orthogonal to the leading edge and, unlike the dorsal cables, terminated with focal adhesion markers and thus represent stress fibers (fig. S8) (19). The number of dorsal cables near nuclei increased during nuclear movement (30 to 90 min) (Fig. 2B and fig. S7).

Live imaging of actin filaments labeled with Lifeact-mCherry (ibidi, Munich, Germany) (22), which did not perturb actin, centrosome orienta-

tion, or nuclear positioning (fig. S9), also showed dorsal and ventral actin cables near the nucleus (Fig. 2, C and D). After LPA stimulation, dorsal cables and the nucleus moved rearward at the same rate ($0.35 \pm 0.10 \mu\text{m}/\text{min}$) (Fig. 2, C to E, and movie S4). Ventral stress fibers remained stationary or shortened slightly (Fig. 2C, fig. S10, and movie S5), suggesting that nuclear movement may be driven by the dorsal cables. Nesprin2G-depleted cells formed well-organized dorsal cables, which moved at the same rate ($0.35 \pm 0.13 \mu\text{m}/\text{min}$) as in control cells, yet nuclei in nesprin2G-depleted fibroblasts barely moved ($0.06 \pm 0.09 \mu\text{m}/\text{min}$) (Fig. 2, D and E, and fig. S11). Thus, nesprin2G is not required for the organization or retrograde transport of dorsal cables.

In nesprin2G-depleted cells rescued with GFP-mini-N2G, linear structures that colocalized with dorsal cables were detected in the nuclear envelope (Fig. 3A). Endogenous nesprin2G was also observed in linear arrays that colocalized with dorsal cables in LPA-stimulated cells (Fig. 3A). This localization of GFP-mini-N2G required functional CH domains (fig. S12) and actin cables, because actin or myosin II inhibitors prevented their formation (Fig. 3B). Fluorescence recovery after photobleaching revealed that the mobility of GFP-mini-N2G in the linear arrays was reduced compared with that in the bulk nuclear membrane (fig. S13).

SUN2 was the only other nuclear protein (among SUN1, SUN2, LBR, lamin A/C, and lamin

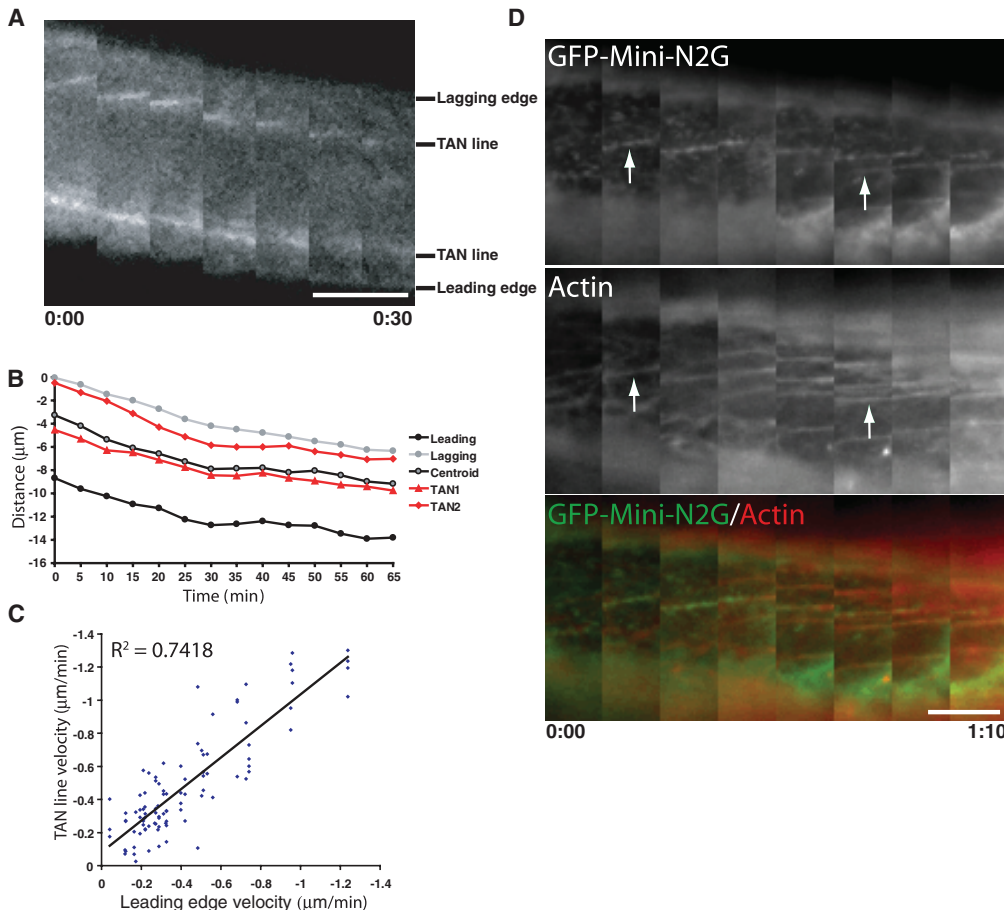


Fig. 4. TAN lines are coupled with dorsal actin cables during nuclear movement. **(A)** Fluorescence kymograph of GFP-mini-N2G TAN lines in a nucleus from a nesprin2G-depleted cell from movie S6. The leading and lagging edges of the nucleus and two TAN lines are indicated. Each image is 5 min. **(B)** Representative distance versus time plot of TAN lines, nuclear centroid, and leading and lagging nuclear edges from a movie of a cell treated as in (A). **(C)** Comparison of GFP-mini-N2G TAN line and leading nuclear edge velocities in cells treated as in (A). Data are from 30 movies of 30 cells. R^2 is the coefficient of determination. **(D)** Fluorescence kymograph of GFP-mini-N2G and Lifeact-mCherry on the dorsal nuclear surface of a nucleus in a cell treated as in (A) from movie S7. Arrows indicate examples of TAN lines forming on actin cables. Images are every 10 min and oriented with the wound edge at the top. Time is hour:min for (A) and (D). Scale bars in (A) and (D), 5 μm .

B1) that colocalized with linear arrays of GFP-mini-N2G (Fig. 3C and fig. S14). Expressed GFP-SUN2 also formed linear arrays on the dorsal surface of nuclei (fig. S15). Because the linear arrays are actin-dependent, specific molecular assemblies of nesprin2G and SUN2 and not deformations of the nuclear envelope, we refer to them as TAN (transmembrane actin-associated nuclear) lines.

SUN2 depletion also inhibited nuclear positioning and centrosome orientation (Fig. 3D and figs. S4 and S16). Unlike other nuclear envelope proteins examined, nesprin2G levels and nuclear localization were reduced by SUN2 depletion (fig. S4). Expressed GFP-SUN2, but not GFP-SUN1, rescued the polarity defects in SUN2-depleted cells. These results indicate that SUN1 and SUN2 are not functionally equivalent for nuclear movement (Fig. 3D and fig. S16). GFP-mini-N2G expression in SUN2-depleted cells failed to restore centrosome orientation and nuclear positioning, further suggesting that nesprin2G requires SUN2 for normal nuclear movement (Fig. 3D and fig. S16).

During nuclear movement, GFP-mini-N2G TAN lines were observed moving rearward with the nucleus and dorsal actin cables labeled with Lifeact-mCherry (Fig. 4, A to D, fig. S17, and movies S6 and S7). Velocity measurements confirmed this correlation for many moving nuclei (Fig. 4C). Dual imaging of GFP-TAN lines and Lifeact-mCherry additionally revealed that TAN lines formed after dorsal cables (Fig. 4D, arrows). Thus, actin organizes TAN lines, which may form to functionally harness the force of retrograde actin flow for nuclear movement.

Centrosome orientation has been implicated in directed cell migration (5, 16). We determined whether inhibition of centrosome orientation by disrupting nuclear movement affected cell migration. Nesprin2G- or SUN2-depleted cells migrated into in vitro wounds slower than control cells did, consistent with previous results (fig. S18, A and B) (12). Furthermore, wound-edge cells expressing RFP-KASH fell behind the wound edge compared with cells expressing control constructs (fig. S19, C and D). Thus, the LINC complex and nuclear movement is required for efficient cell migration.

Here, the LINC complex components nesprin2G and SUN2 were found to assemble into TAN lines that provide a direct linkage between the nucleus and retrograde moving dorsal actin cables. Because each component of the TAN lines (nesprin2G, SUN2, and actin cables) was required for nuclear movement and TAN lines moved with dorsal cables during nuclear movement, we suggest that this assembly transmits force from retrograde actin flow to the nucleus. The accumulation of multiple nesprin2G and SUN2 molecules along an actin cable may be necessary to resist forces exerted by retrograde actin flow. Analogous force-resisting mechanisms were proposed for yeast KASH and SUN protein arrays, although these appear as spotwelds and anchor microtubules to the nuclear envelope (23).

We propose that TAN lines are functionally analogous to focal adhesions, which are clustered

transmembrane integrins and associated cytoplasmic proteins that link the extracellular matrix to actin filaments. Both structures assemble in response to actin bundling by nonmuscle myosin II, and both transmit force across membranes. Unlike focal adhesions, TAN lines span two membranes and form along the length of an actin cable. Because INM SUN2 does not directly contact cytoplasmic dorsal actin cables, a regulatory “outside-in” signaling pathway, analogous to that for integrins (24), may exist that allows SUN2 to recognize nesprin2G engaged with actin and assembled into TAN lines. Recent work in mice suggests that nesprins and SUNs are important for normal mammalian development (13, 25, 26). It will be interesting to explore whether these proteins need to form a macromolecular structure like TAN lines to function during development.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5994/956/DC1
Materials and Methods

Figs. S1 to S18

Tables S1 to S3

References

Movies S1 to S7

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mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists

Nanxin Li, Boyoung Lee, Rong-jian Liu, Mounira Banasr, Jason M. Dwyer, Masaaki Iwata, Xiao-Yuan Li, George Aghajanian, Ronald S. Duman*

The rapid antidepressant response after ketamine administration in treatment-resistant depressed patients suggests a possible new approach for treating mood disorders compared to the weeks or months required for standard medications. However, the mechanisms underlying this action of ketamine [a glutamate N-methyl-D-aspartic acid (NMDA) receptor antagonist] have not been identified. We observed that ketamine rapidly activated the mammalian target of rapamycin (mTOR) pathway, leading to increased synaptic signaling proteins and increased number and function of new spine synapses in the prefrontal cortex of rats. Moreover, blockade of mTOR signaling completely blocked ketamine induction of synaptogenesis and behavioral responses in models of depression. Our results demonstrate that these effects of ketamine are opposite to the synaptic deficits that result from exposure to stress and could contribute to the fast antidepressant actions of ketamine.

Major depressive disorder (MDD) afflicts about 17% of the population and is one of the leading causes of total disability

and economic burden (1). Although there are medications that alleviate depressive symptoms, these have serious limitations. Most notably, available

treatments require weeks or months to produce a therapeutic response, and only about one-third of patients respond to the first medication prescribed (2). In contrast, recent studies demonstrate that a single, low dose of a glutamate N-methyl-D-aspartic acid (NMDA) receptor antagonist produces a rapid (within hours) antidepressant response that lasts

for up to 7 days (3, 4) and is effective in MDD patients who are resistant to traditional antidepressants (5). The mechanisms underlying rapid antidepressant actions are likely more complicated than simple NMDA receptor blockade and so far have not been identified. We carried out a series of studies to examine the cellular signaling pathways that mediate the behavioral actions of NMDA receptor blockade, focusing on signaling cascades known to rapidly influence synaptic plasticity (6).

The drug used for clinical trials is ketamine, a nonselective NMDA receptor antagonist (7). A low dose of ketamine (10 mg/kg), which is re-

ported to have antidepressant actions in behavioral models of depression (7), rapidly activated the mammalian target of rapamycin (mTOR) signaling pathway in the prefrontal cortex (PFC) of rats (Fig. 1A). Activation of mTOR signaling was observed in a preparation enriched in synaptoneurosomes (fig. S1) and included increased levels of the phosphorylated and activated forms of eukaryotic initiation factor 4E binding protein 1 (4E-BP1), p70S6 kinase (p70S6K), and mTOR (Fig. 1A). Increased phosphorylation of 4E-BP1, p70S6K, and mTOR is transient, returning to basal levels by 2 hours after ketamine administration (Fig. 1A). In contrast, other antidepressants

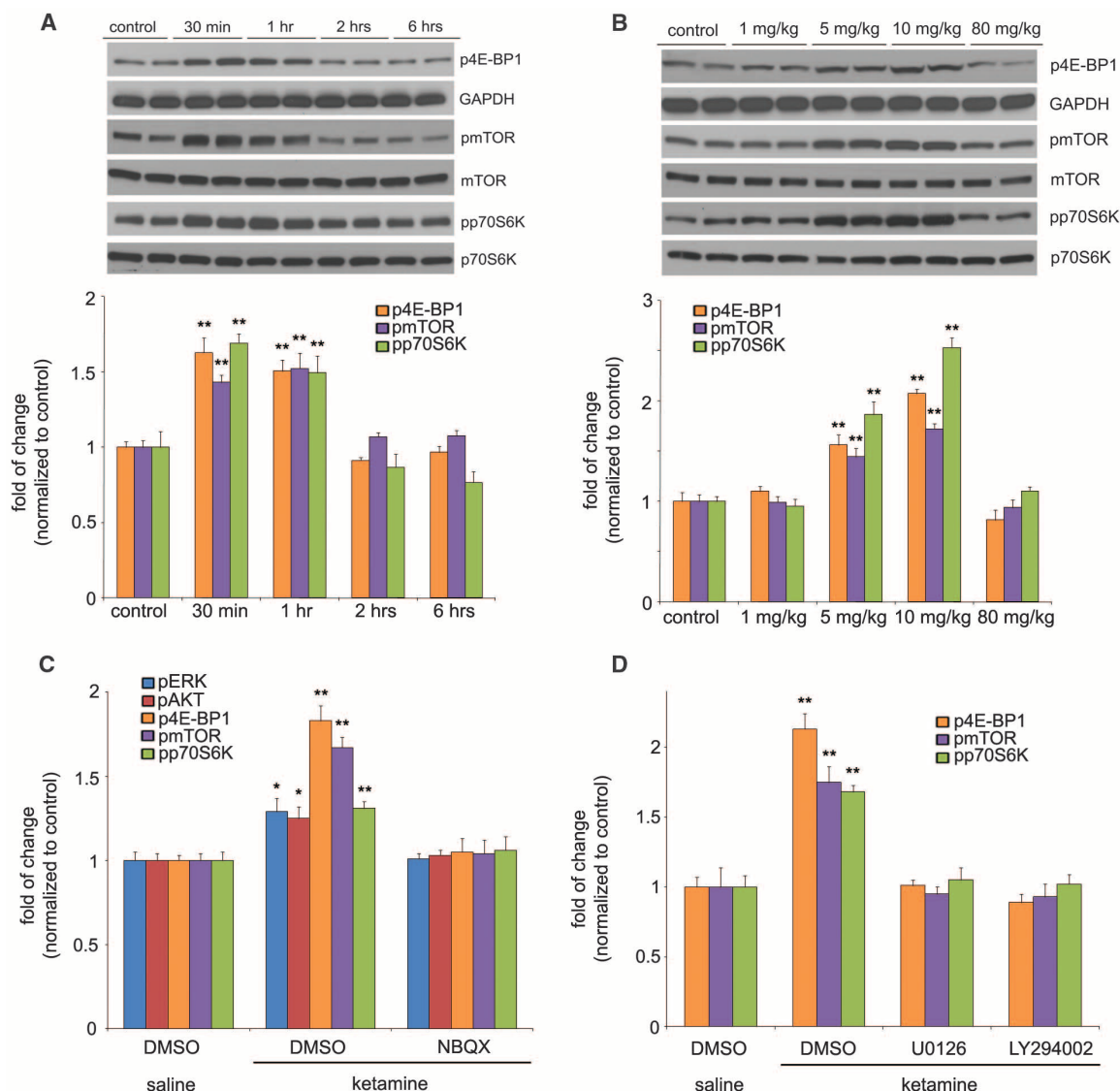


Fig. 1. Ketamine transiently and dose-dependently activates mTOR signaling in rat PFC. Values represent mean $\pm$ SEM [$n = 4$ animals; * $P < 0.05$; ** $P < 0.01$; analysis of variance (ANOVA)]. (A) Time course of ketamine [10 mg/kg, intraperitoneally (ip)]-induced mTOR signaling determined by Western blot analysis of phospho-mTOR (pmTOR), phospho-4E-BP1 (p4E-BP1), and phospho-p70S6K (pp70S6K) in synaptoneurosomes of PFC. Levels of total mTOR, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and p70S6K were also determined. (B) Dose-dependent activation, determined 1 hour after ketamine administration, of pmTOR,

p4E-BP1, and pp70S6K. (C) Pretreatment (10 min) with NBQX (10 mg/kg, ip) blocked ketamine (10 mg/kg, ip) activation of pmTOR, p4E-BP1, and pp70S6K, as well as upstream signaling kinases phospho-ERK (pERK) and phospho-Akt (pAkt) (analyzed 1 hour after ketamine). Levels of pERK1 and pERK2 were similarly regulated and were combined for quantitative analysis. DMSO, dimethyl sulfoxide. (D) Pretreatment (30 min) with inhibitors of ERK (U0126, 20 nmol, ICV) or PI-3k/Akt (LY294002, 20 nmol, ICV) abolished ketamine (10 mg/kg, ip) activation of mTOR signaling proteins (analyzed 1 hour after ketamine administration).

tested, including electroconvulsive seizure, imipramine, or fluoxetine, did not significantly influence mTOR signaling (fig. S2). Ketamine produced a similar rapid and transient increase in the phosphorylated and activated forms of extracellular signal-regulated kinase (ERK, including ERK1 and ERK2) and protein kinase B (PKB/Akt) (fig.

S3A), growth factor signaling pathways that have been linked to activation of mTOR signaling (8). The activation of 4E-BP1, p70S6K, mTOR (Fig. 1B), ERK, and Akt (fig. S3B) was dose dependent, occurring at relatively low doses (5 to 10 mg/kg) that produce antidepressant behavioral actions but not at a higher anesthetic dose (7).

The antidepressant actions of ketamine have been reported to require glutamate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptors (7). In line with this, we found that pretreatment (10 min) with a selective AMPA receptor inhibitor, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX),

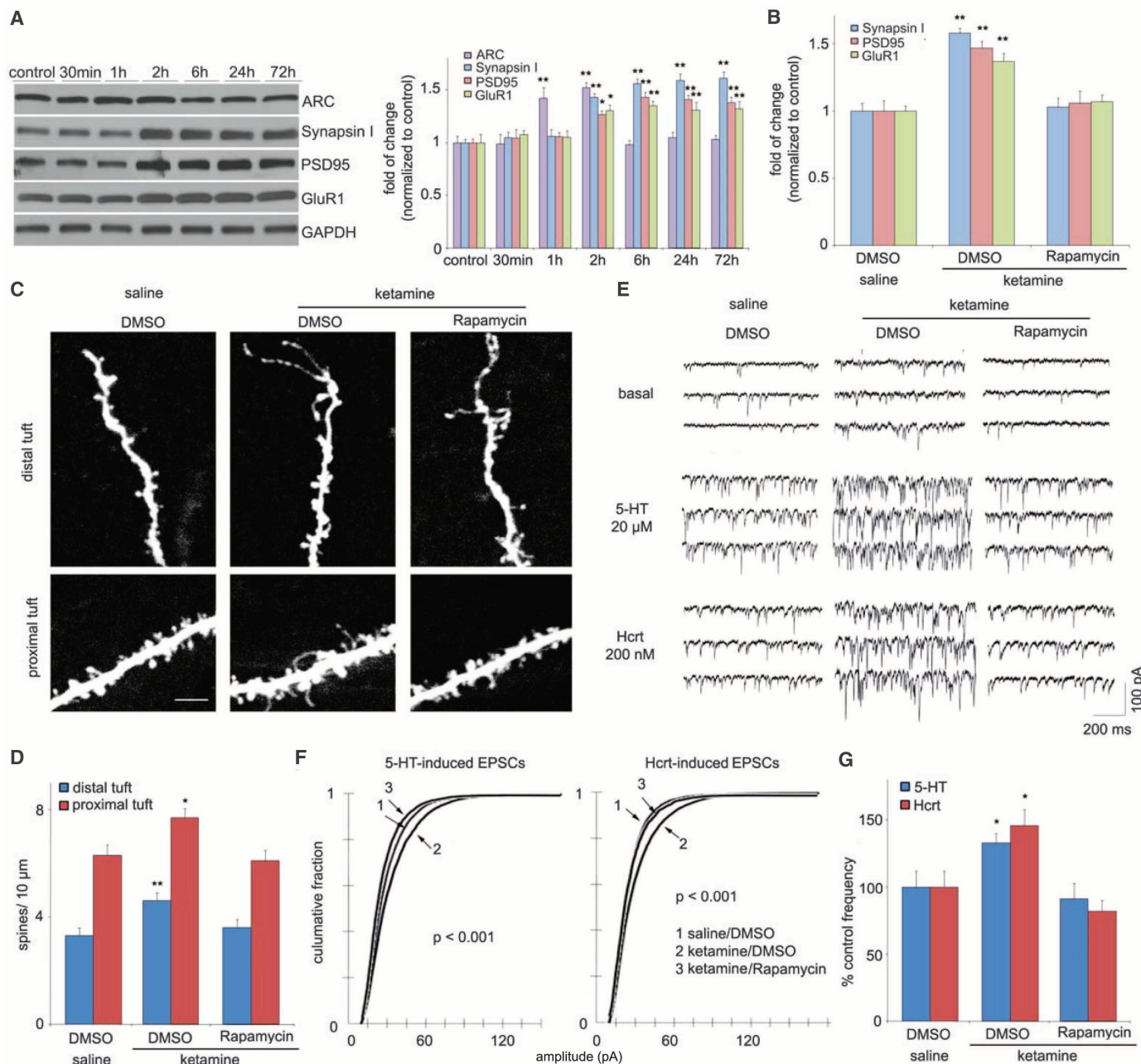


Fig. 2. Ketamine rapidly increases synaptic proteins and spine number. **(A)** Time course for ketamine (10 mg/kg, ip) induction of synaptic proteins Arc, synapsin I, PSD95, and GluR1 in synaptoneurosomes of PFC and **(B)** blockade by pretreatment (30 min) with a selective mTOR inhibitor, rapamycin (0.2 nmol, ICV) (values represent mean $\pm$ SEM, $n = 4$ to 6 animals; $*P < 0.05$; $**P < 0.01$; ANOVA). **(C)** Ketamine-increased spine density in mPFC, analyzed by two-photon microscopy 24 hours after treatment. Representative images are shown of high-magnification Z-stack projections of apical tuft segments of neurobiotin-labeled layer V pyramidal cells (scale bar indicates 5 μ m). **(D)**

Results are the mean $\pm$ SEM (eight cells from four rats in each group; $*P < 0.05$; $**P < 0.01$, t test). **(E)** Ketamine enhanced mPFC layer V pyramidal cell EPSC responses. Sample whole-cell voltage-clamp recordings of 5-HT and hypocretin-induced EPSCs in slices (24 hours after Ketamine treatment). **(F)** Cumulative probability distributions showing significant increases in amplitude ($P < 0.0001$, Kolmogorov-Smirnov test- z value = 6.5 for 5-HT and 6.7 for Hcrt) and **(G)** frequency of 5-HT- and hypocretin-induced EPSCs ($n = 12$ neurons per group; $*P < 0.05$, t test). Ketamine induction of spine density and function were blocked by rapamycin infusions [(C) to (G)].

completely blocked the induction of phosphorylated 4E-BP1, p70S6K, and mTOR, as well as ERK and Akt in the PFC (Fig. 1C and fig. S4). We assessed the possible involvement of ERK and Akt pathways in the activation of mTOR by intracerebroventricular (ICV) infusion of specific pharmacological antagonists. Inhibition of either ERK (U0126) or Akt [via inhibition of the upstream Akt activator phosphatidylinositol (PI3)-kinase by LY294002] blocked ketamine induction of phos-

phorylated 4E-BP1, p70S6K, and mTOR (Fig. 1D and fig. S5).

Activation of mTOR has been functionally linked with local protein synthesis in synapses, resulting in the production of proteins required for the formation, maturation, and function of new spine synapses (8). To determine whether ketamine induction of mTOR produces similar effects, we analyzed levels of several synaptic proteins in synaptoneurosomes from PFC. Ketamine

administration increased levels of the postsynaptic proteins PSD95 and GluR1, as well as the presynaptic protein synapsin I (Fig. 2, A and B). Ketamine-induced increases in these synaptic proteins was delayed relative to ERK, Akt, and mTOR, peaking at 2 to 6 hours, but remained elevated up to 72 hours. Ketamine also induced expression of activity-regulated cytoskeletal-associated protein (Arc), with an intermediate (peak at 1 to 2 hours) but transient time course. The func-

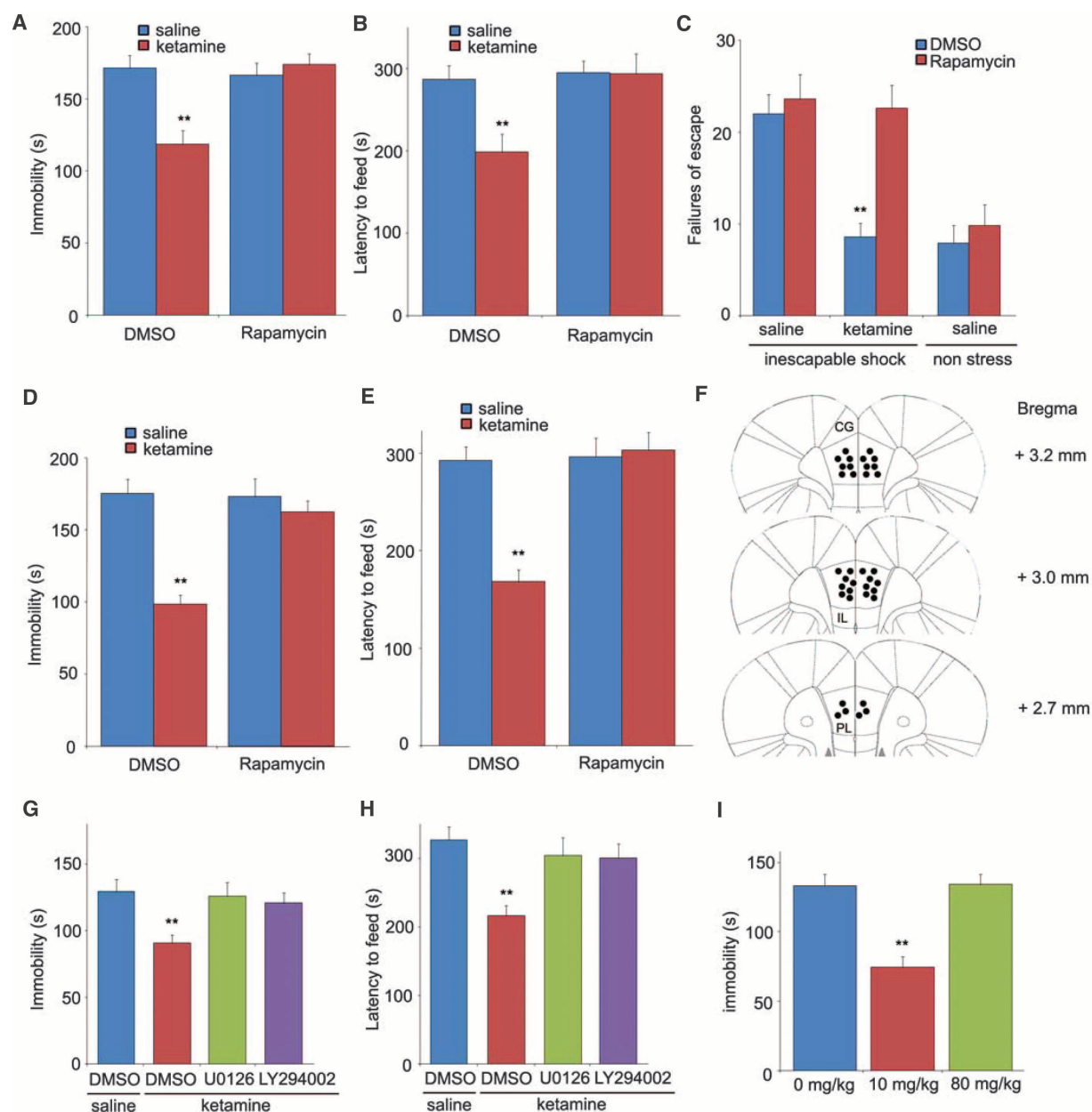


Fig. 3. Rapid behavioral actions of ketamine require mTOR signaling. Rapamycin was infused (0.2 nmol, ICV) 30 min before ketamine (10 mg/kg, ip), and responses in the FST (A), NSFT (B), or LH (C) paradigms were determined. Values represent mean $\pm$ SEM ($n = 6$ to 8 animals; $*P < 0.05$; $**P < 0.01$; ANOVA). Infusion of rapamycin (0.01 nmol) into the mPFC blocked the antidepressant actions of ketamine (10 mg/kg, ip) in the FST (D) and NSFT (E). (F)

Location of rapamycin infusions in the PFC (infusion sites are the same on the right and left sides because of the use of bilateral cannulae). Pretreatment with inhibitors of ERK (U0126, 20 nmol, ICV) or PI3 kinase/Akt (LY294002, 20 nmol, ICV) blocked the behavioral effects of ketamine in FST (G) and NSFT (H). (I) Low (10 mg/kg) but not a high (80 mg/kg) anesthetic dose of ketamine produced an antidepressant action in the FST.

tional connection between mTOR activation and synaptic protein synthesis was examined by ICV infusion of selective mTOR inhibitor rapamycin, which completely blocked the induction of PSD95, GluR1, and synapsin I (Fig. 2B and fig. S6).

An increase in synapse-associated proteins indicates that ketamine administration rapidly increases synapse and spine formation. We therefore examined the influence of ketamine on spine number and morphology by two-photon imaging of the apical tuft of prelabeled layer V medial PFC (mPFC) pyramidal neurons. Analysis of the images shows that ketamine administration increased spine density in both distal and proximal segments of the apical tuft 24 hours after ketamine injection (Fig. 2, C and D). Ketamine also increased the population of mushroom (large-diameter) spines (fig. S7), an indication of increased spine maturation and synaptic strengthening (9). Synaptic strengthening is supported by analysis

of the electrophysiological properties of the same cells, demonstrating that excitatory postsynaptic current (EPSC) frequency responses to serotonin [5-hydroxytryptamine (5-HT)], which are mediated predominantly by cortical-cortical synapses in the apical tuft (10), are significantly increased by ketamine administration (Fig. 2, E to G). Responses to hypocretin and orexin, which occur selectively at apical thalamocortical synapses (11), are also increased (Fig. 2, E to G). In both cases, there is a significant increase in EPSC amplitude (Fig. 2F), consistent with the elevation in mushroom spines (fig. S7) and GluR1. Ketamine induction of spines and 5-HT and hypocretin-stimulated excitatory postsynaptic potentials (EPSPs) are blocked by infusion of rapamycin (Fig. 2, C to G, and fig. S7).

We next determined whether activation of mTOR signaling is required for the antidepressant behavioral actions of ketamine. As previously reported, a low dose of ketamine produced a

rapid antidepressant effect in two well-established behavioral models of despair, the forced swim test (FST) and learned helplessness (LH) in response to inescapable stress (IES), as well as a model of anxiety, the novelty suppressed feeding test (NSFT) (Fig. 3, A to C). Traditional antidepressants are effective in these tests after acute (1 day, FST), subchronic (6 days, LH), and chronic (21 days, NSFT) treatment, whereas a single dose of ketamine was effective in all three tests. Moreover, infusion (ICV) of rapamycin completely abolished the antidepressant actions of ketamine in all three tests (Fig. 3, A to C). The role of mTOR signaling in the actions of ketamine was further examined by local infusion of rapamycin into the mPFC, which resulted in a complete blockade of the behavioral effects of ketamine in FST and NSFT tests (Fig. 3, D and E) (location of rapamycin infusions into the mPFC is shown in Fig. 3F). The effects of inhibitors of ERK and PI3k/Akt, which mediate ketamine induction of mTOR, were also examined. Infusion (ICV) of selective ERK (U0126) or PI3k/Akt (LY294002) inhibitors blocked the antidepressant actions of ketamine in the FST and NSFT (Fig. 3, G and H). Lastly, the behavioral actions of ketamine in the FST occurred with a dose-response profile similar to that for induction of ERK, Akt, and mTOR (Fig. 3I). This addresses a major point of discussion in the field as to whether an anesthetic dose of ketamine could produce an antidepressant effect.

Basic research and clinical studies demonstrate that stress and depression can cause atrophy of PFC pyramidal neurons in rodent models and in postmortem tissue of depressed subjects (10, 12), and brain-imaging studies report a decrease in PFC volume in depressed patients (13). Exposure to stress in the LH paradigm decreased levels of synaptic proteins GluR1, PSD95, and synapsin I, and a single dose of ketamine reversed this deficit in a rapamycin-sensitive manner (fig. S8). These results indicate that ketamine induction of synapse-associated protein synthesis provides a mechanism for rapid reversal of stress- and/or depression-mediated deficits and could enhance PFC-mediated connectivity and function.

Ketamine is a psychotomimetic drug with abuse potential, and a more selective agent would be desirable for clinical antidepressant use. There have been reports that selective NMDA receptor subunit 2B (NR2B) antagonists produce antidepressant actions similar to ketamine in both preclinical models and clinical trials (7, 14). Therefore, we examined the influence of Ro 25-6981, a NR2B-selective compound, on mTOR signaling and rapamycin-sensitive behaviors. Ro25-6981, at a dose (10 mg/kg) identified in the FST to be optimal (fig. S9), produced a transient activation of mTOR signaling (increased phosphorylation of 4E-BP1, p70S6K, and mTOR), as well as ERK and Akt signaling in synaptoneurosome from PFC (Fig. 4, A and B). Ro 25-6981 administration also increased levels of the synaptic proteins Arc, PSD95, GluR1, and synapsin I in synaptoneurosome (Fig. 4, A and

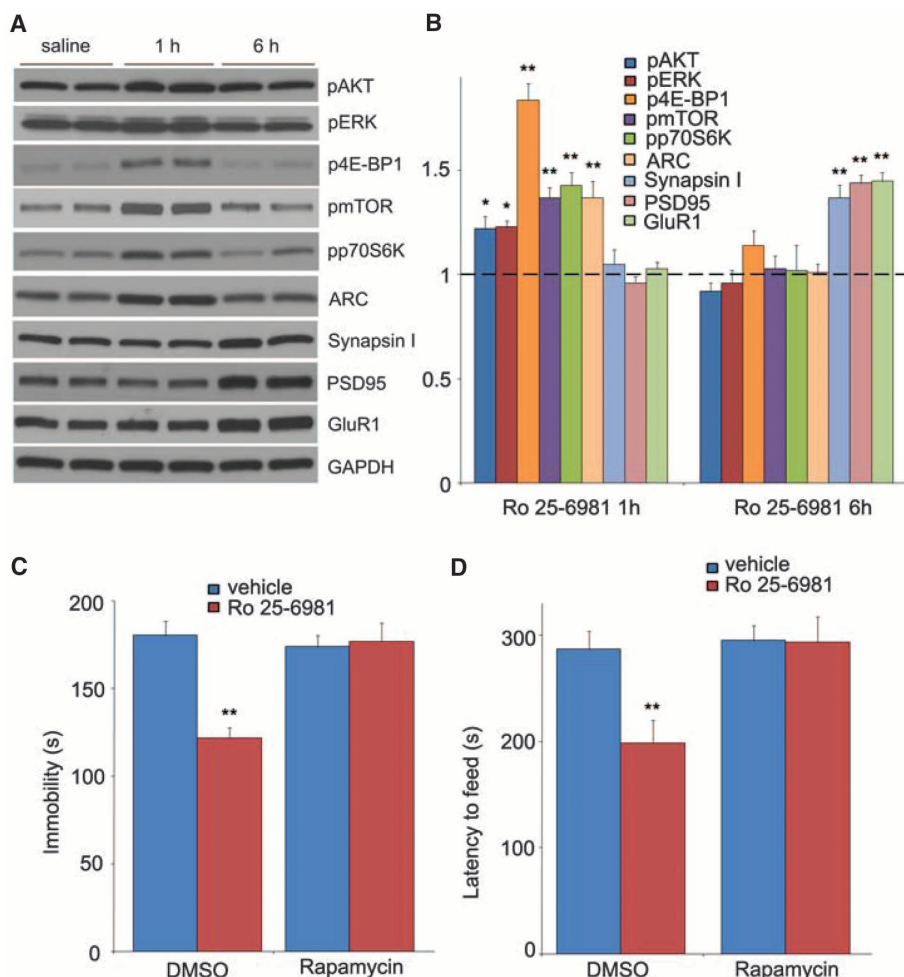


Fig. 4. Selective NR2B antagonist Ro 25-6981 activates mTOR signaling and produces rapamycin-sensitive behavioral effects. (A and B) Effects of Ro 25-6981 (10 mg/kg, ip) on pmTOR, p4E-BP1, pp70S6K, pERK, pAkt, Arc, synapsin I, PSD95, and GluR1 in PFC. (C and D) Pretreatment with rapamycin (0.2 nmol, ICV) abolished the actions of Ro 25-6981 in FST (C) and NSFT (D). Values represent mean $\pm$ SEM ($n = 6$ to 8 animals; * $P < 0.05$; ** $P < 0.01$; ANOVA).

B). Induction of Arc was more rapid and transient, whereas PSD95, GluR1, and synapsin I were delayed, similar to the delay observed after ketamine administration. Lastly, Ro 25-6981 administration produced rapid antidepressant effects (24 hours) in the FST and NSFT, which were blocked by preinfusion (ICV) of rapamycin (Fig. 4, C and D).

Fast activation of mTOR signaling resulting in rapid and sustained elevation of synapse-associated proteins and spine number in the PFC represents a mechanism for the rapid antidepressant actions of ketamine. These effects result in elevated 5-HT neurotransmission, a primary target of traditional antidepressants, although the latter can take weeks or months to induce a therapeutic response (2). The mechanisms underlying the induction of mTOR signaling are unclear, but the requirement for glutamate-AMPA receptor activation is consistent with the hypothesis that there is a subset of NMDA receptors, possibly on γ -aminobutyric acid (GABA)-releasing interneurons,

that when antagonized, block GABA release lead to disinhibition of glutamate signaling (15). Further characterization of these actions of NMDA receptor blockade and the signaling pathways that stimulate mTOR signaling and mediate the rapid induction of synapses in the PFC will provide novel therapeutic targets for antidepressant drug development.

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Supporting Online Material

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Females Use Multiple Mating and Genetically Loaded Sperm Competition to Target Compatible Genes

Sarah R. Pryke, Lee A. Rollins, Simon C. Griffith*

Individuals in socially monogamous species may participate in copulations outside of the pair bond, resulting in extra-pair offspring. Although males benefit from such extra-pair behavior if they produce more offspring, the adaptive function of infidelity to females remains elusive. Here we show that female participation in extra-pair copulations, combined with a genetically loaded process of sperm competition, enables female finches to target genes that are optimally compatible with their own to ensure fertility and optimize offspring viability. Such female behavior, along with the postcopulatory processes demonstrated here, may provide an adaptive function of female infidelity in socially monogamous animals.

In socially monogamous species, optimal female mate choice is constrained by the number of high-quality or compatible social partners available. Extra-pair sexual relationships provide an opportunity for females to ensure against the fitness costs of genetically incompatible social partnerships and to improve the genetic quality of at least some of their offspring by mating with extra-pair males that are either genetically more compatible or of higher genetic quality than their social partner (1–3). To date, studies investigating the potential additive genetic benefits provided by extra-pair males have generally either failed to

detect effects or have reported only weak or inconsistent results (4–6). However, most studies have inferred female infidelity through the forensic molecular analysis of the outcome of fertilization (i.e., paternity), and with few exceptions (7), have not considered female copulatory behavior, including the number of partners and the distribution of copulations among them. Therefore, it has been difficult to gain insight into the underlying postcopulatory processes, and ultimately, to determine the adaptive function of female polyandry in socially monogamous animals.

In the socially monogamous Gouldian finch (*Erythrura gouldiae*), genetic incompatibility between interbreeding red and black head-color morphs (that is, different genotypes) results in high offspring mortality rates (more than 60% greater mortality from egg to sexual maturity than compatible pairs of the same head color) (8).

Mate compatibility is signaled by head color, which is determined by a Z-linked gene: Females are hemizygous for this gene (Z^r black, Z^R red), whereas male genotypes can be homozygous Z^rZ^r (black), Z^RZ^R (red), or heterozygous Z^RZ^r (red). Individuals demonstrate precopulatory mate preferences for their own morph type; however, perhaps because of constraints on preferred-mate availability, up to 30% of breeding pairs in wild populations are mixed morph (9).

We tested for adaptive female participation in extra-pair copulations by presenting captive female Gouldian finches breeding in genetically compatible (social partner of the same genotype) and incompatible social pairs (social partner of different morph), with an opportunity to seek an extra-pair copulation with either a compatible or incompatible male (10). On the day after the female-initiated egg laying (day 1; day 0 = day the first egg is laid), the birds in the social pair were physically and visually separated by an opaque divider, which split the cage in half, and a virgin male in breeding condition was placed with the female for 60 min. In the context of selection for compatible genes (1), this experimental design provided a predicted adaptive context (incompatible social partner and compatible extra-pair partner), a maladaptive context (compatible social partner and incompatible extra-pair partner), and two selectively neutral situations (both social and extra-pair partners either incompatible or compatible) (Fig. 1).

Despite morph-assortative mate preferences (9) and selection against mixed morph mating (11), surprisingly, females across all experimental contexts were equally likely to engage in extra-pair behavior [$\chi^2 = 0.87$, degrees of freedom (df) = 3, $P = 0.83$]. Overall, 77.5% (31 of 40) of females copulated with the extra-pair male (Fig. 1). All

Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.

*To whom correspondence should be addressed. E-mail: simon.griffith@mq.edu.au

successful copulations (i.e., cloacal contact) were preceded by active female solicitation (that is, female orientates cloaca toward male and tail quivers). Only two extra-pair males attempted to force copulations in the 40 trials (5%); females successfully resisted both (i.e., no cloacal contact) and never solicited to these sexually aggressive males. Similarly, forced copulation attempts by social mates were rare (0.6%; 9 of 1398 recorded copulations) and also unsuccessful.

Using characteristic courtship displays (9), potential extra-pair males displayed to females in 95% of the trials (38 of 40 trials). Displays were initiated rapidly [1.71 ± 1.76 (SEM) minutes within introduction to the cage], and males did not discriminate between compatible and incompatible females [i.e., no differences in initiation of singing and courtship behaviors; analysis of variance (ANOVA), $F_{1,38} = 0.39$ to 0.76 , $P = 0.37$ to

0.54]. In six trials, females ignored male displays and did not solicit copulations; in one trial a female solicited, but no successful copulation followed. Most females quickly solicited to extra-pair males (5.32 ± 6.18 min from introduction of the male and within 1.48 ± 1.93 minutes from initiation of male courtship display), irrespective of their own head color (ANOVA, $F_{1,32} = 0.15$, $P = 0.67$) or the extra-pair males' compatibility (head color; ANOVA, $F_{1,32} = 1.98$, $P = 0.18$) or display behaviors (ANOVA, $F_{1,32} = 0.08$ to 1.80 , $P = 0.14$ to 0.78). By participating in extra-pair copulations, extra-pair males can increase their reproductive success with limited investment. In contrast, although a female's participation in extra-pair copulations with a compatible extra-pair male increases the number of eggs she produces [generalized linear model (GLM), $\chi^2 = 3.78$, $P = 0.03$], the female bears the cost of this additional investment, which

may also potentially jeopardize the investment provided by her social partner (12–14). Females may solicit extra-pair copulations with incompatible males because they are unable to always accurately assess male compatibility [for example, red females cannot differentiate between phenotypic red males that are homozygous (compatible) or heterozygous (incompatible) (15)].

Because birds can store sperm from different males for an extended time within their reproductive tract (16), we next examined the potential for postcopulatory sexual selection. As predicted for the outcome of sperm competition for compatible genes (1, 17), the 31 successful extra-pair copulations resulted in a disproportionate number of fertilized offspring among broods ($\chi^2 = 10.10$, $df = 3$, $P = 0.02$) (Fig. 1). Furthermore, after controlling for differences in brood size between the multiple-paternity contexts (Fig. 2), more offspring per brood were fathered by the extra-pair male in the adaptive context than in the two neutral contexts (GLM, $\chi^2 = 12.32$, $P < 0.001$).

Due to the timing of his copulation, the extra-pair male was unable to fertilize the first and second egg, which would have already been fertilized by the social mate; thus, sperm competition between the social and extra-pair males was limited to one to seven potentially fertilizable eggs (i.e., the third to ninth eggs). Within the 14 mixed-paternity clutches, the proportion of extra-pair offspring changed with laying order (GLM, $\chi^2 = 21.2$, $P = 0.004$) (Fig. 2). Similar to other avian studies (18, 19), extra-pair males gained disproportionate fertilization success 24 to 48 hours after copulating, despite females copulating just once (1.28 ± 0.61 ; range = one to two copulations) with most extra-pair males (91.2%) and even though females were in continual contact with their social mates and frequently copulated with them (34.67 ± 7.69 from day -5 to 9), both before (16.22 ± 2.27) and after (18.45 ± 5.45) the experimental extra-pair copulation (Fig. 2). Furthermore, after the extra-pair opportunity (that is, when the social pairs were reunited), 39 of the 40 social males copulated with their female at least once within 30 min of being reunited.

Consistent with the lower value placed on reproductive effort when breeding with incompatible social partners (11), females copulated less frequently with incompatible social mates (1.87 ± 0.14) than with compatible partners (2.75 ± 0.91 ; ANOVA, $F_{1,39} = 6.05$, $P = 0.002$). In particular, females in incompatible social pairings copulated less frequently with their social mates after participating in extra-pair copulations with compatible extra-pair males (i.e., adaptive context; $t = 1.92$, $n = 9$, $P = 0.05$). Nevertheless, the reduced number of social copulations does not explain the greater extra-pair fertilization efficacy (during the period of sperm competition; 2 to 9 days) in this context (Fig. 2C). Compared with the neutral contexts, extra-pair copulations resulted in significantly more extra-pair offspring in the adaptive context ($\chi^2 = 48.43$, $P < 0.001$), and significantly fewer in the maladaptive context ($\chi^2 = 43.21$, $P < 0.001$), than expected from

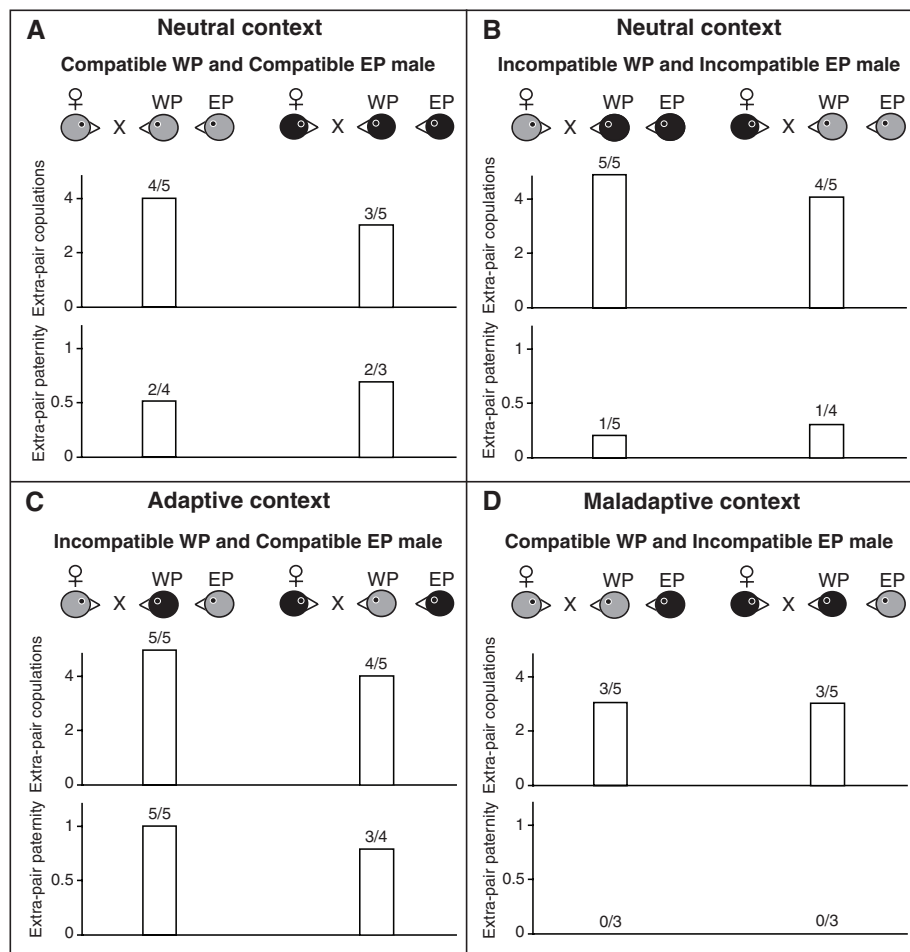


Fig. 1. Across the four experimental contexts, most red (left bars) and black (right bars) females in compatible and incompatible social pairs pursued extra-pair copulations with both compatible and incompatible males. However, resulting fertilization success was highly biased: (A) Four of seven broods and (B) two of nine broods contained extra-pair offspring when both social and extra-pair males were compatible or incompatible; (C) eight of nine broods contained extra-pair offspring when the social male was compatible and extra-pair male was incompatible; and (D) none of the six broods contained extra-pair offspring when the social male was compatible and the extra-pair was male incompatible. WP, within-pair (social) mate; EP, extra-pair mate.

the relative number of extra-pair and social copulations (fig. S1).

We were unable to account for strategic male allocation of insemination size with respect to female quality (19, 20). However, in contrast to previous allocation studies (20), extra-pair males showed equal (and rapid) propensity to mate with both compatible and incompatible females, and virgin extra-pair males (housed in single-sex cages since fledging) were given only this single opportunity to copulate with a female (thus, trade-offs in sperm allocation in lieu of future matings seem unlikely). Although differences in ejaculate size by extra-pair and social males may, in part, explain the disproportional fertilization success of the extra-pair males, the considerable differential

postcopulatory success by extra-pair males across the different contexts demonstrates the extent to which genetic compatibility can bias the outcome of fertilization (Fig. 2).

Although what, and even if, females gain from participating in extra-pair copulations has been debated (4–6), Gouldian finch females seeking extra-pair copulations may ensure fertilization of their eggs by a compatible male and produce more viable offspring. In line with previous results (8), offspring were more likely to survive (from egg to sexual maturity) if their parents were genetically compatible [generalized linear mixed model (GLMM), $\chi^2 = 62.12$, $P < 0.001$], irrespective of whether they were extra-pair or within-pair offspring (GLMM, $\chi^2 = 1.47$, $P = 0.44$). For

females with genetically incompatible social mates, extra-pair copulations with compatible males results in a 38.9% increase in offspring survival, compared to females forced to mate exclusively with their genetically incompatible social mates (8).

Although this system is unusual because of the extent of within-species genetic incompatibility, similar processes may allow females to avoid the negative effects of inbreeding (21–23) and hybridization (24, 25) or to target the positive benefits of heterosis (26). This is likely to be particularly important in systems in which females cannot phenotypically or behaviorally assess compatibility. The cryptic nature of extra-pair copulations (27), as well as the discrepancy between observed extra-pair copulation behavior and the resulting extra-pair paternity, may explain why the adaptive function of female participation in extra-pair copulations has remained elusive (5, 6, 28). The postcopulatory processes demonstrated here may also help to explain previous studies showing variation in intra- and interspecific extra-pair paternity rates (2, 13) and weak or inconsistent differences in quality between extra-pair and within-pair offspring (4, 28). Our findings suggest that, to understand the evolution of female polyandry in socially monogamous animals, we need to account for both pre- and postcopulatory processes. Quantifying all extra-pair copulations and their timing, as well as the strength of selection on genetic compatibility, is challenging. However, our results suggest that neglecting these details may underestimate the extent of behavioral infidelity and the potential adaptive benefits to females.

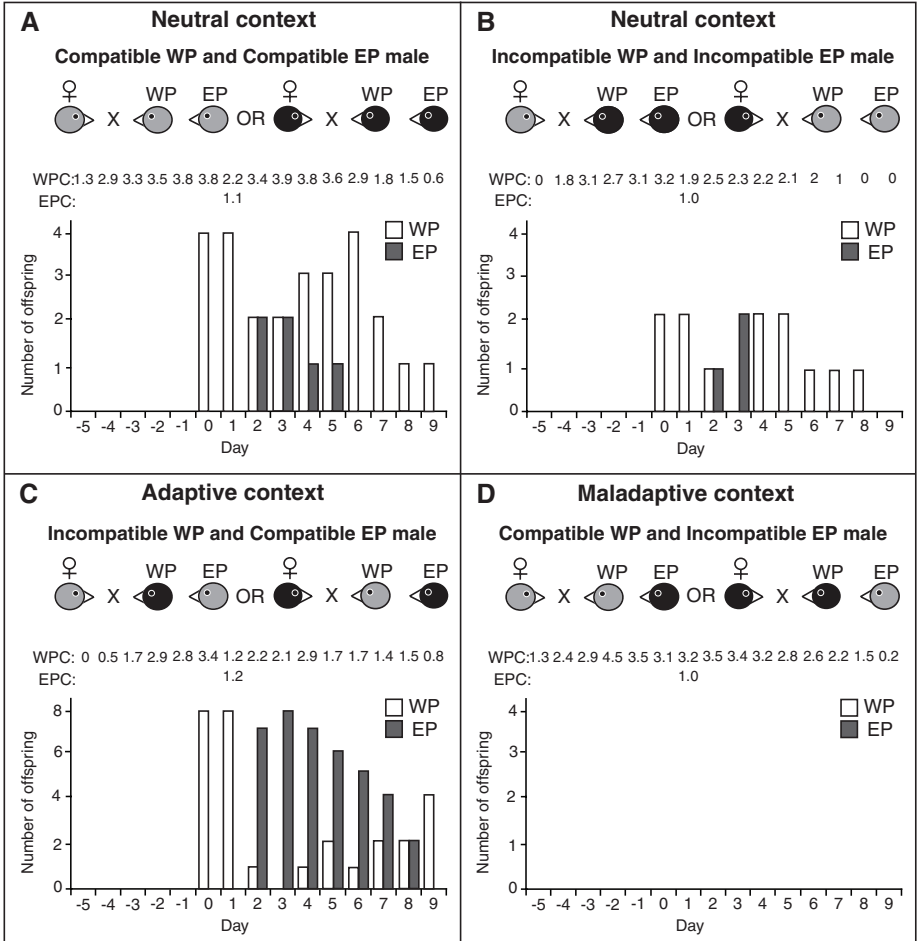


Fig. 2. Extra-pair paternity is determined by the relative genetic compatibility of the WP and EP male with the female. In each predicted context (A to D), the number of eggs fertilized by the social and extra-pair male is shown in laying order, and the average number of copulations each day is provided (above the graph). Conservatively, given that females may store sperm from their social mate (from day –5 to day 1), when both the social and extra-pair males were (A) compatible or (B) incompatible, 4.1 to 5.8% of copulations by the extra-pair male resulted in (A) 28.6% and (B) 27.3% of the offspring produced from the potentially fertilizable eggs (days 2 to 9; extra-pair males were unable to fertilize eggs laid on days 1 and 2). In contrast, (C) 6.1% of copulations by compatible extra-pair males resulted in 76.5% of the offspring produced when the social mate was incompatible, whereas (D) 3.9% of extra-pair copulations by incompatible extra-pair males resulted in no fertilized eggs when the social mate was compatible. WPC, within-pair copulations; EPC, extra-pair copulations.

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Supporting Online Material

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Fig. S1
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Cell Lineage Reconstruction of Early Zebrafish Embryos Using Label-Free Nonlinear Microscopy

Nicolas Olivier,^{1*} Miguel A. Luengo-Oroz,^{2*} Louise Duloquin,^{3*} Emmanuel Faure,⁴ Thierry Savy,⁴ Israël Veilleux,¹ Xavier Solinas,¹ Delphine Débarre,¹ Paul Bourguine,^{4,5} Andrés Santos,² Nadine Peyrieras,^{3,6†} Emmanuel Beaurepaire^{1†}

Quantifying cell behaviors in animal early embryogenesis remains a challenging issue requiring in toto imaging and automated image analysis. We designed a framework for imaging and reconstructing unstained whole zebrafish embryos for their first 10 cell division cycles and report measurements along the cell lineage with micrometer spatial resolution and minute temporal accuracy. Point-scanning multiphoton excitation optimized to preferentially probe the innermost regions of the embryo provided intrinsic signals highlighting all mitotic spindles and cell boundaries. Automated image analysis revealed the phenomenology of cell proliferation. Blastomeres continuously drift out of synchrony. After the 32-cell stage, the cell cycle lengthens according to cell radial position, leading to apparent division waves. Progressive amplification of this process is the rule, contrasting with classical descriptions of abrupt changes in the system dynamics.

Although classical developmental biology is characterized by qualitative descriptions, recent work underlines the requirements for precise measurements to enable formal reconstruction integrating the genetic, molecular, and cellular levels of organization (1–3). The optimization of microscopy imaging techniques and improved data algorithmic processing are key issues in such reconstructions. Parallelized linear microscopy such as light-sheet fluorescence microscopy provides fast imaging but suffers from loss of information with depth (4). Point-scanning two-photon microscopy provides deeper imaging (5) but exhibits slower frame rate, compromising

automated individual cell tracking in whole organisms (6). Furthermore, the usual implementation of these two paradigms does not allow homogeneous illumination in spherical samples, leading to a difficult tradeoff between the detection of deep structures and illumination-induced perturbation in outer layers. Finally, relying on fluorescent staining of biological structures brings additional artifacts and limitations. Exploiting the intrinsic optical nonlinear properties of the sample is a valuable, although challenging, alternative. Second-harmonic generation (SHG) is obtained from dense noncentrosymmetric structures such as oriented microtubule assemblies (7–9), including mitotic spindles (8, 10). Third-harmonic generation (THG) is obtained from optical heterogeneities (11)—such as the interface between an aqueous medium and a lipidic, mineralized, or absorbing structure (12)—and allows morphological imaging of small organisms (10, 13).

Here, we show that combining SHG and THG imaging of unlabeled embryos with a scanning scheme matching embryo morphology provides adequate three-dimensional (3D) imaging over time for the automated reconstruction of cell behavior during zebrafish embryo cleavage stages (14). Ad hoc image analysis strategies for cell position, division, and shape identification were used to produce a complete and validated lineage

tree for a cohort of six zebrafish embryos until the 1000-cell stage, annotated with minute-level division timing, micrometer-accuracy cell coordinates, and shape characteristics. These data provided a quantitative spatiotemporal description of the wave-like division cycles and allowed the construction of a prototypic digital blastula. The cycle duration of sister cells exhibited variability that did not correlate with cell volume, revealing unexpected cell division asynchrony and asymmetry from the first division cycles and leading to increasing cell heterogeneity by the time of midblastula transition (MBT) (14).

An appropriate image acquisition scheme was devised to provide high-resolution time-lapse imaging of intrinsic SHG and THG signals (Fig. 1 and supporting online material). Excitation in the 1.2- μ m range reduced nonlinear endogenous absorption by the sample and allowed simultaneous two-photon-excited fluorescence (2PEF) imaging of red fluorescent proteins (Fig. 1, B and C) for control experiments. When imaging a spherical embryo, scattering and aberrations typically result in reduced signal at the center of each plane (Fig. 1E). We therefore scanned each plane of a half-sphere along a spiral trajectory with variable speed to spend more time imaging the innermost cells (Fig. 1, D to F, and fig. S1). This conformal strategy provided optimal acquisition time and minimal photoperturbation (fig. S2). SHG and THG signals were co-optimized by using rotating linear incident polarization. In addition, because THG contrast from a specific structure depends on its size relative to the focal volume (15), moderate focusing (3.5- μ m Z-resolution) was used to highlight cell interface compared with smaller subcellular structures (Fig. 2D).

Combining the conformal scanning scheme described above, sensitive detection, infrared excitation wavelength, and appropriate focusing and polarization conditions allowed homogenous detection of mitotic spindles and cell and tissue phenotypic features in the whole unlabeled zebrafish embryo during cleavage stages. The blastoderm was contained in a half sphere of 440- μ m radius imaged with a temporal resolution of 80 s and a volumetric pixel size of 2 by 2 by 4 μ m, suitable for further automated reconstruction of the cell lineage tree.

The intrinsic THG signal revealed a number of structures and dynamic processes (Fig. 2, A to J, and movies S1 to S6) and highlighted cell contours even better than membrane staining by

¹Laboratory for Optics and Biosciences, Ecole Polytechnique, CNRS, INSERM, Palaiseau, France. ²Biomedical Image Technologies, Universidad Politécnica de Madrid, and Biomedical Research Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Madrid, Spain. ³Neurobiologie et Développement, Institut de Neurobiologie Alfred Fessard, CNRS, Gif/Yvette, France. ⁴Centre de Recherche en Epistémologie Appliquée, Ecole Polytechnique, CNRS, Paris, France. ⁵Réseau National des Systèmes Complexes, 57-59 rue Lhomond, Paris, France. ⁶Institut des Systèmes Complexes Paris Ile-de-France, 57-59 rue Lhomond, Paris, France.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: nadine.peyrieras@inaf.cnrs-gif.fr (N.P.); emmanuel.beaurepaire@polytechnique.edu (E.B.)

farnesylated mCherry, indicating the presence of a sizeable intercellular space as corroborated by numerical simulations (Fig. 2, K to M). Higher-numerical aperture THG imaging produced signal discontinuities or double interfaces at cell boundaries (Fig. 2D), indicating local distance variation between cells. This interpretation is reinforced by the absence of THG signal from the lateral junction of the enveloping layer (EVL) (fig. S3). THG also allowed visualizing trafficking phenomena, including cell membrane completion at the 32-cell

stage (movie S2) and nuclear envelope dynamics during mitosis (movie S3). In addition, when embryos were overilluminated, photo damage correlated with enhanced intracellular THG signal, serving as a control of embryo physiological condition (fig. S2). THG also revealed yolk lipid platelets interface and allowed visualizing the phenomenology of cytoplasmic streams during the first cell cycles (movie S1). Combining THG/SHG/2PEF in *βactin:H2B/mcherry* transgenic embryos (16) allowed correlating SHG and THG signals with

mitosis phases (Fig. 2, G to I, and movie S4). As shown in Fig. 2O, SHG intensity exhibited a nearly Gaussian temporal profile peaking at metaphase, providing measurement for division timing and cell cycle duration with subminute accuracy.

We designed an original image processing methodology for automatically extracting cell position, membrane geometry, and cell lineage, taking advantage of mitosis metasynchrony and limited cell displacements during the first 10 division cycles (fig. S4 and movies S5 and S6).

Fig. 1. Conformal scanning harmonic microscopy scheme. (A) Schematized zebrafish embryo imaged from the animal pole. (B) Sample mounting for upright microscopy; excitation (Exc.) and detection geometry (2PEF epidetected, THG-SHG transmitted). (C) Energy diagrams and wavelengths involved. (D) Lateral position (left) and depth (right) of the focal point inside a spherical sample when using raster scanning (top) versus conformal scanning (bottom). (E) THG imaging with raster (top) and spiral scanning (bottom). Acquisition time is 5 s. Scale bar, 100 μm . (F) XZ two-photon excitation using raster (top) and conformal (bottom) scan patterns with preferential energy delivery to the deepest regions in the conformal scheme.

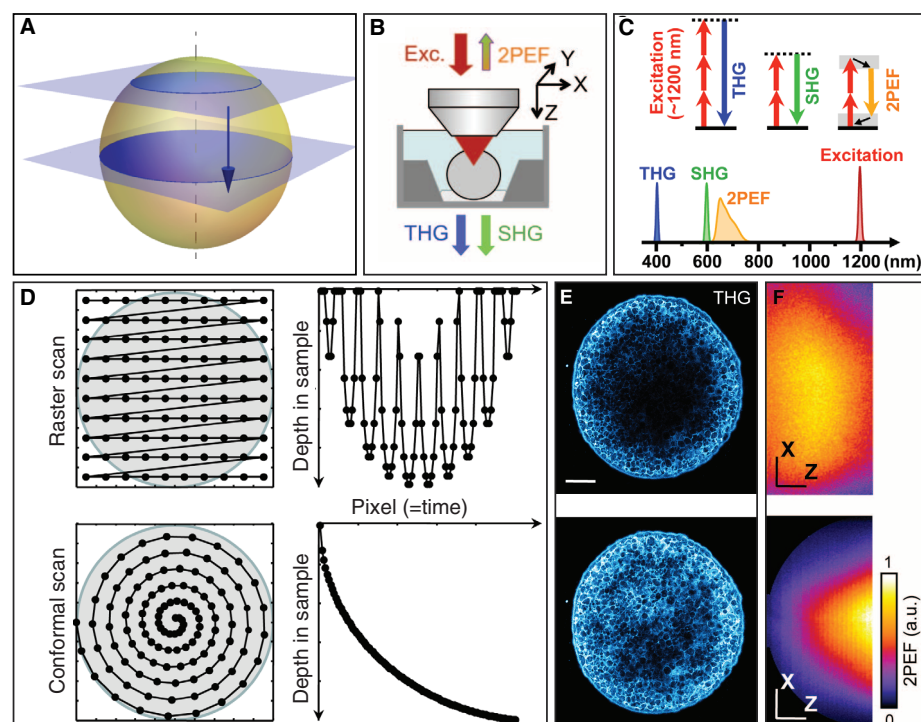
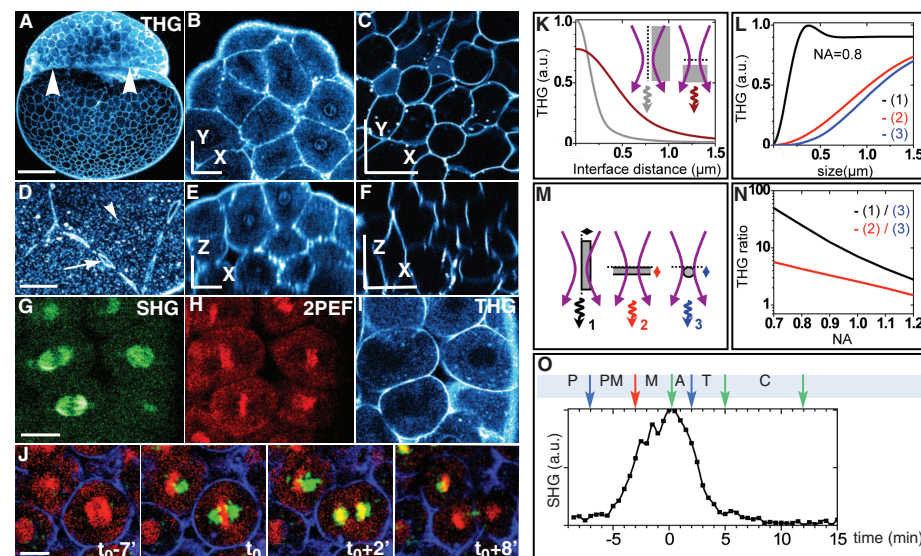


Fig. 2. THG and SHG signals in the zebrafish embryo during cleavage stages. (A) Sagittal THG image, 512-cell stage, animal pole to the top; white arrowheads indicate yolk-blastoderm interface. Scale bar, 200 μm . (B and C) THG XY image and (E and F) ZX projection. (B and E) Blastoderm cells imaged with 0.8 numerical aperture (NA) excitation, 64-cell stage. Scale bars, 50 μm . (C and F) Yolk platelets imaged under conditions similar to B and E. (D) THG XY image of blastoderm cells with 1.2 NA excitation; white arrow points to double interface, arrowheads point to intracellular organelles. Scale bar, 30 μm . (G to I) *βactin:H2B/mcherry* transgenic imaged with SHG-2PEF-THG. Scale bar, 20 μm . (J) Merged SHG-2PEF-THG, temporal sequence. (K) Calculations of THG from lateral (gray) and axial (brown) interfaces as a function of position, modeling the nuclear membrane signal. (L to N) Calculations of THG from heterogeneities, modeling cell boundaries (black, red) and vesicles (blue). (N) THG ratio between a 0.6- μm slab and a 0.4- μm diameter sphere as a function of NA. Using moderate NA, enhanced cell interfaces signal compared to small organelles. (O) Evolution of SHG signal intensity during mitosis. Prophase, P; prometaphase, PM; metaphase, M; telophase, T; and cytokinesis, C, revealed by THG-SHG-2PEF signals (movie S4).



This allowed measurements along the cell lineage tree throughout cleavage (17) until the onset of MBT (18). Mitoses were detected from the

SHG channel by compressing the data into 10 volumes corresponding to cell cycles and detecting in each volume the expected number of SHG

spots corresponding to cell divisions (2, 4, 8...) (Fig. 3A and movie S7). A distance rule respecting the symmetric and limited displacement of

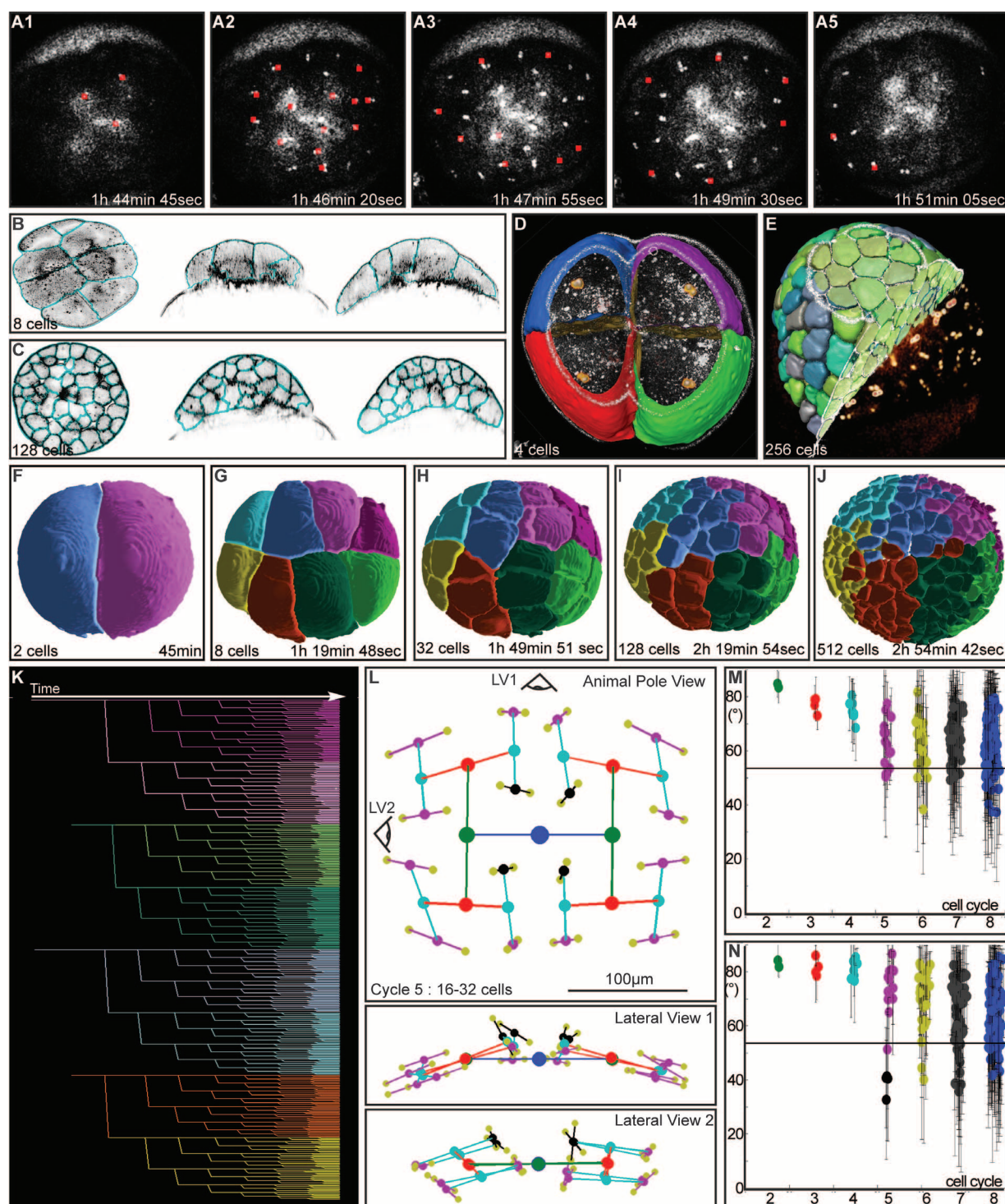


Fig. 3. Automated reconstruction of the cell lineage tree for a cohort of zebrafish early blastulas. (A) SHG spot detection (red) overlaid to XY projections of SHG images; A1 to A5 encompass cell cycle 5 (with cell division 1 at 45 min); see movie S8. (B and C) THG image segmentation at the 8-cell stage (B) and 128-cell stage (C); see movie S9. (D and E) Reconstruction of 4-cell stage (D) and 256-cell stage (E) embryos. In white, raw THG signal; orange, detected SHG spots; colors, segmented cell contours. (F to J) Digital embryo from 1-cell stage to 512-cell stage. See movie S10. (K) Flat representation of

the lineage tree with eight-cell stage clones; same colors as in (G) to (J). (L) Spatial deployment of the cell lineage up to cycle 5. (Top) Animal pole view. (Bottom) Lateral views. See movie S11 and fig. S6. (M) Angle between successive sister cells dipoles, in degrees. 55° (black line) corresponds to random orientation. (N) Angle between sister cells dipoles and the normal to the embryo surface. Cells divided tangentially to the embryo surface until cycle 5 (16 to 32 cells). At cycle 5, the four central blastomeres [shown in black in (L) and (N)] divided orthogonally to the surface.

daughter cells after mitosis was then used to link consecutive sequences. Finally, accurate cell cycle timing was obtained by fitting time-dependent SHG intensity to a Gaussian function (Fig. 2O). The lineage tree validation is a critical step as tracking errors propagate along the tree. Validation was performed using an interactive visualization interface that allowed comparing raw and reconstructed data at each cell cycle before processing the next one (movie S8). This strategy

provided error-free lineage trees with minimal human intervention (fig. S5). Cell shapes were then extracted from THG images by using cells' spatial coordinates as seeds to perform a region growing-based contour detection. A rough shape approximation was first obtained by building a Voronoi diagram, and membrane detection was refined using a viscous watershed algorithm (19) (movie S9 and Fig. 3, B to E). Data sets from six different embryos were processed to obtain the

corresponding digital blastulas (Fig. 3, fig. S5, and movies S10 to S13).

The phenomenological reconstruction allowed a systematic analysis of spatiotemporal correlations between cell position, cell cycle duration, mitosis duration, cell volume, and cell division orientation. Cells during cycles 1 to 4 were constrained by the absence of basal plasma membrane contacting the yolk, and divisions displayed the known stereotyped orientation with

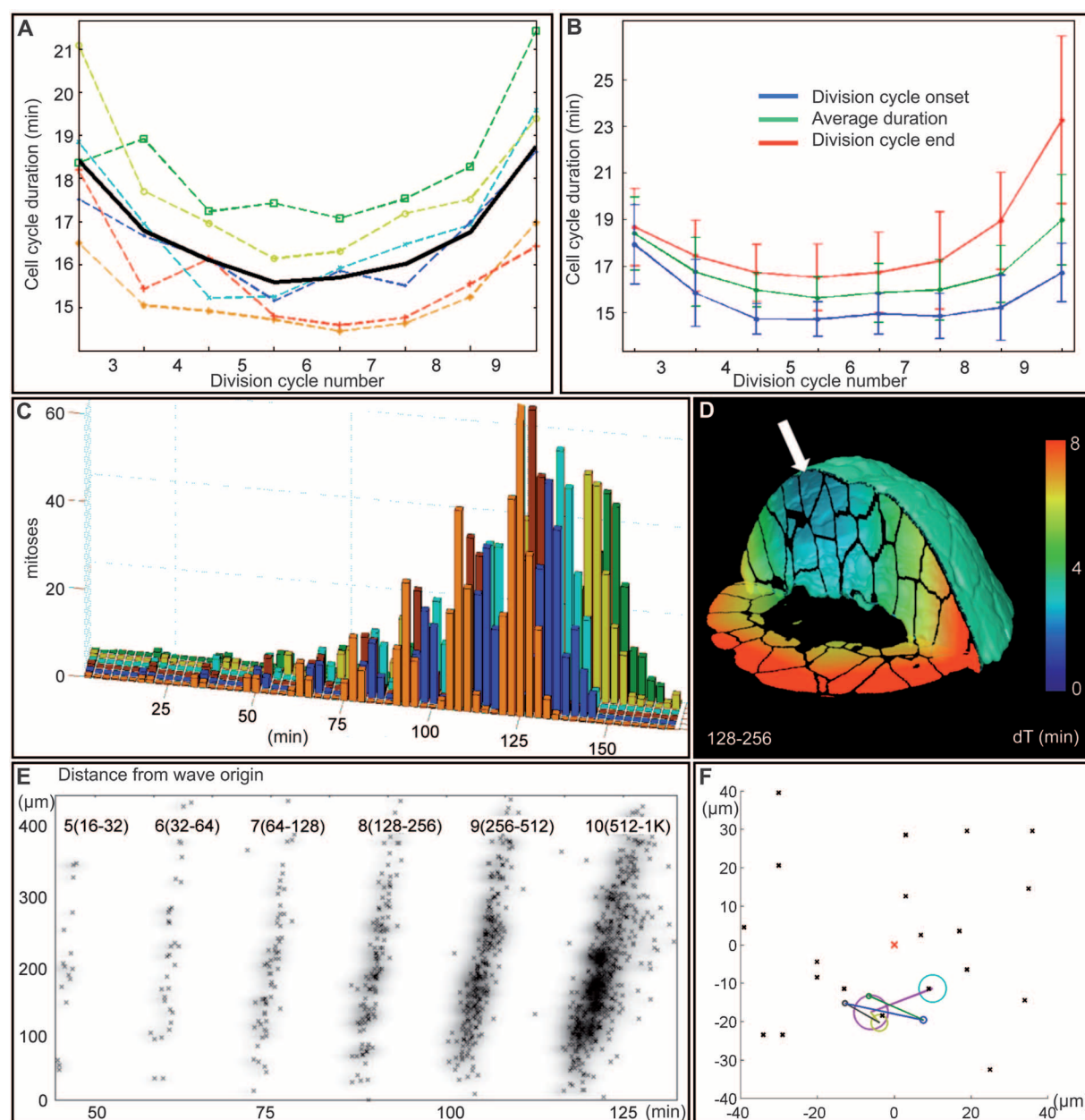


Fig. 4. Data statistical homogeneity, local features, and global patterns. (A) Mean cell cycle duration (metaphase-to-metaphase) from cycles 2 to 10 for six embryos (black line, average). (B) Mean cell cycle duration (green) compared with cycle duration at the beginning (blue) and at the end (red) of the division cycle, averaged for six embryos. (C) Temporal histogram of mitoses in six embryos. (D) Spatiotemporal representation of the mitotic pseudo-wave at

cycle 8; time ranges from 0 min (blue, cycle onset) to 8 min (red, cycle end); white arrow points to the pseudo-wave origin. (E) Scatter plot of mitosis time (SHG peak at metaphase) as a function of distance to the pseudo-wave origin. (F) XY projection of the position of the pseudo-wave origin for cycles 5 to 10 with respect to the animal pole (red cross) and other mitoses at cycle 10 (black crosses). Data for five other embryos are in the supporting online material.

little fluctuation from one embryo to the other (Fig. 3M). Division orientation at cycle 5 started exploring one more spatial direction at 90° relative to the previous division, thus allowing the formation of a partially double-layered blastoderm (Fig. 3, L and N). This quantitative observation contrasts with the classical description presenting the 32-cell stage as a single cell layer (14). From divisions 5 to 8, larger fluctuations occurred in division orientation, resulting in progressively more random orientations (Fig. 3N and fig. S6). By averaging and registering six different embryos, we constructed a prototype of the lineage spatiotemporal deployment (fig. S6 and movie S10). From cycle 3 to cycle 9, cell displacements were limited to cytokinesis, and displacement speed from one division to the other remained constant (fig. S7), suggesting that biochemical dynamics (20, 21) do not impact cell displacements until MBT, quantitatively supporting previous observations (18). Consistently, the clones corresponding to the first eight blastomeres observed at the 512-cell stage remained clustered (Fig. 3, F to J, and movie S10).

In the temporal domain, the main dynamical change was the evolution of cell cycle duration as previously observed (22). The cell cycle shortened until division 5 (18.5 ± 1.5 min to 15.5 ± 1 min), followed by lengthening until division 10 (up to 18.5 ± 1.5 min) (Fig. 4A). The minimum value correlated with plasma membrane completion at the 32-cell stage, suggesting a specific regime for the division of the large early blastomeres (23, 24). Continuous cell cycle lengthening thereafter is hypothesized to result from the degradation of maternal products and S-phase lengthening (21). Furthermore, as early as the 4-cell stage, cell divisions did not occur synchronously, and the delay measured between the first and last division at each cell cycle increased continuously through cleavages (Fig. 4, B and C).

In addition, after the 32-cell stage, cell cycle lengthening increased with the physical distance from the blastoderm surface (Fig. 4, D and E, and figs. S8 to S11), so that in the particular case of sister cells, the deeper tended to divide later than her sister located closer to the surface (fig. S13). We hypothesize that this feature correlates with deposition of maternal components by cytoplasmic streams (movie S1) that might have produced a concentration gradient. In any case, fluctuation in sister cells' division timing and steady lengthening in cell cycle duration as a function of cell position relative to the blastoderm surface are sufficient to explain the progressive appearance of a global wave-like pattern of radial division (Fig. 4, D and E). Calculating the position of this pseudo-wave origin relative to the animal pole indicated an early symmetry breaking variably amplified in some cases (fig. S14). A discussion of the cell division patterns during cleavage stages must also take into account the formation of the yolk syncytial layer (YSL) (25). We observed that YSL nuclei typically individualized at the 512-cell stage and underwent three

division cycles with an average timing of 20 to 30 min and displaying a peripheral pattern, thus differing from the radial pattern observed in the blastoderm (movies S14 to S16 and figs. S15 and 16). YSL formation is known to display phenotypic variability (26), but extreme cases of artifactual YSL formation were observed in embryos mechanically constrained by the mounting medium (movie S16 and fig. S17). Altogether, our observations suggest that there is no switch from a radial toward a peripheral pseudo-wave pattern in the blastoderm, in contrast to a recent proposal by Keller *et al.* (4).

Cell volume measurements indicated that up to cycle 10, the nucleocytoplasmic ratio (N/C) was unlikely to trigger cell cycle lengthening as we observed cell volume variability, including random fluctuation between sister cells, with no correlation to cycle duration (figs. S11 and S12). According to other studies, N/C becomes an important factor at later developmental stages (18). Indeed, a pause in cell cycle, regulated by N/C, is described as one of the most prominent features of the MBT (27). This means that by MBT, the N/C variability generated earlier could act as a local fluctuation factor, breaking the global pseudo-wave pattern. Cell cycle lengthening, both globally and as a function of cell position, is the main parameter that should be taken into account for further modeling zebrafish embryo cleavage morphogenesis. Continuity and amplification of the process are the rule, rather than the classically described abrupt changes from synchrony to metasyncrony and asynchrony (14).

Harmonic time-lapse microscopy provides in toto imaging, enabling automated and validated reconstruction of the zebrafish embryo lineage tree during the cleavage period. Because the reconstruction scheme is based on cell cycle metasyncrony and limited cell displacements, we provide a quantitative assessment that cell intrinsic motility does not arise before cell cycle 10, described as the MBT stage (18). The framework presented here automates the phenomenological reconstruction of animal early embryogenesis without the use of fluorescent staining. Conformal acquisition with excitation power and scanning speed matching specimen morphology makes point-scanning multiphoton microscopy an excellent strategy for deep embryo imaging. We anticipate that combining harmonic and fluorescent signals in conformal scanning time-lapse microscopy will provide optimal data sets to achieve the automated tracking of cell trajectories and cell divisions throughout vertebrate embryogenesis. Single-cell analysis along the lineage tree is necessary to provide relevant spatial and temporal correlations underlying emerging patterns. Most of developmental biology still relies on visual inspection and manual work to achieve limited cell tracking, and recent studies have provided only a global estimation of cell movements. In this context, we expect our framework to establish standards in terms of measurements precision and accuracy of automated algorithmic

segmentation and tracking procedures. The quantitative phenomenological reconstruction of the zebrafish embryo cleavage period provided here should serve as a reference for further analysis of the interplay between nano- (molecular and genetic) and macro- (biomechanics) level dynamics.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5994/967/DC1
Materials and Methods

Figs. S1 to S17

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Movies S1 to S16

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c/o Cindy Hutson Rolfe
Department of Cell Physiology and Molecular
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ASSISTANT PROFESSOR Biological Sciences

We are seeking a tenure-track Assistant Professor in the Department of Biological Sciences, College of Environment and Life Sciences (CELS) with research interests in the effects of global change on population-level evolutionary processes. The anticipated appointment is August 1, 2011. Visit [website: http://jobs.uri.edu](http://jobs.uri.edu) to view complete details for job posting #6000230. Position is open until filled, with review of applications to begin October 1, 2010. Only electronic applications will be accepted. Documents to attach in PDF format to your letter of application: curriculum vitae which, through your record of experience, education, publications, research plan, and statement of teaching philosophy, demonstrates your potential for excellence in teaching and for developing a high quality, nationally recognized and externally-funded research program. Additionally, send copies of up to three published papers and arrange to have three letters of reference sent by October 1, 2010 to: **Dr. Brad A. Seibel, Associate Professor, CELS-Bio, Center Biotech Life Science, University of Rhode Island, Kingston, RI 02881.** Visit the department website: <http://www.uri.edu/cels/bio/> for additional information. *The University of Rhode Island is an Affirmative Action/Equal Employment Opportunity Employer and values diversity. URI is an E-Verify Employer.*

A POSTDOCTORAL POSITION is immediately available in the laboratory of **Gerald M. Carlson** at the University of Kansas Medical Center, Kansas City, Kansas, to study the multi-subunit enzyme phosphorylase kinase (PhK). A main goal is to understand the function of the individual subunits of PhK, particularly how single missense mutations in regulatory subunits lead to glycogen storage diseases. Candidates should have a Ph.D. in a biological science, preferably in biochemistry, molecular/cellular biology, or microbiology, and be able to independently plan and execute experiments. Outstanding skills in English are required. Previous experience with molecular cloning, cell culture, and baculovirus-mediated protein expression would be valuable.

To view complete position description and apply online only, go to [website: http://jobs.kumc.edu](http://jobs.kumc.edu) and search for position #J0201147. Please attach a cover letter detailing experience and research interests, along with curriculum vitae and three pertinent references. *University of Kansas is an Equal Opportunity/Affirmative Action Employer.*

Positions NIH

THE NATIONAL INSTITUTES OF HEALTH



Department of Health and Human Services National Institutes of Health National Institute on Alcohol Abuse and Alcoholism

Director, Division of Treatment and Recovery Research (DTRR)



The National Institute on Alcohol Abuse and Alcoholism (NIAAA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (HHS), is recruiting for a senior executive to serve as the Director of the Division of Treatment and Recovery Research (DTRR).

The Director, DTRR, provides national leadership for research on the treatment of alcohol use disorders, including setting scientific priorities through the development of long-term strategic plans and execution of funding decisions. In this capacity, the Director, DTRR leads the Division's efforts on planning, stimulating, developing, and supporting clinical research on cutting-edge therapies for alcoholism. Clinical research at the NIAAA encompasses medications development, behavioral therapies, combined medications and behavioral therapies, recovery research, health services research, and the translation of research into clinical practice. Medications development is one of the NIAAA's top research priorities. The Director, DTRR, oversees the NIAAA's work on the full continuum of research included under medications development—from human laboratory studies to clinical trials, which requires close collaboration with internal and external scientists and researchers with other Federal State and Local government agencies, and national and international research organizations. The Director, DTRR serves as the principal advisor to the Director, NIAAA on alcohol treatment and recovery issues and advises the National Advisory Council on pending grant applications and the status of programs in the federal and private sector.

The selected candidate will be expected to hold a M.D., Ph.D., or equivalent degree. Criteria for selection includes: experience in developing, implementing and/or evaluating behavioral and clinical therapies, specifically in the area of medications development, relevant to alcoholism; experience in managing/leading a complex clinical research organization, experience and expertise in communicating clinical, basic research and programmatic information to scientific and non-scientific audiences; a strong publication record in the field of clinical, behavioral, and medications development research and experience in developing, implementing and managing multidisciplinary and trans-disciplinary research programs on treatments for alcohol use disorders and determinants of post-treatment recovery.

The Director, DTRR, is an Excepted Service position (Title 42), and the successful candidate will be appointed at a salary commensurate with qualifications and experience. Full Federal benefits including leave, health and life insurance, long-term care insurance, retirement, and savings plan (401K equivalent) will be provided.

Interested candidates should submit a curriculum vitae, bibliography, and the names, addresses, contact numbers (phone and fax) and e-mail address of four references by the closing date to the following e-mail account:

E-mail: dttrrrecruit@mail.nih.gov

Applications will be accepted through **September 15, 2010**, or until the position is filled.

National Institute of Neurological Disorders and Stroke



Tenure-Track Investigator

Dense Reconstruction of the Nervous System/Connectomics

The Division of Intramural Research of the National Institute of Neurological Disorders and Stroke (NINDS), NIH has begun an international search for outstanding investigators for tenure-track positions in the area of circuit/system neuroscience. In particular, we seek neuroscientists using high resolution, high-throughput imaging technologies to reconstruct the detailed structure and connectivity of nervous system circuits. The NINDS Division of Intramural Research boasts active basic, translational and clinical research programs in neuroscience, neurological disorders and stroke. These programs operate within the NIH Intramural Research Program, one of the largest and most active basic and clinical research environments in the world, and are a central component of a vibrant neuroscience community at NIH. The successful individual will initiate and direct an independent research program focused on developing and applying high-throughput reconstruction techniques to important problems in circuit function, development or degeneration. The candidate will have earned a PhD, MD or MD/PhD degree and will employ excellent scientific and communication skills to configure an original, productive and collaborative research program. Outstanding candidates who have established internationally recognized highly accomplished research programs may also be considered for a tenured position to build a dynamic, productive research group. Laboratory facilities, shared research facilities, research funds and salary are competitive with premier academic institutions. Applicants should send curriculum vitae, bibliography, statement of research interests, and have three letters of reference sent to: **Dr. Jeffrey Diamond, National Institute of Neurological Disorders and Stroke, c/o Nhuyen Quach, Office of the Scientific Director, Division of Intramural Research, Building 35 Room GA908, NIH, Bethesda, MD 20892 or Nhuyen.Quach@NIH.gov.** Applications will be reviewed beginning **September 15, 2010** and proceed until successful candidates are recruited.



NIEHS National Institute of Environmental Health Sciences National Institutes of Health

Intramural Fellow Position in Molecular Regulation of the Cytochrome P450s Research Triangle Park, North Carolina

A postdoctoral position in the Human Metabolism Group headed by Dr. Joyce Goldstein will become available October 1, 2010. Projects include the transcriptional and epigenetic regulation of the human CYP2C genes.

Qualifications include a doctoral degree (within the last 5 years) in one of the related areas: Molecular biology, genetics, biochemistry, pharmacology. Research experience with molecular biology techniques including transcriptional regulation, cloning, site-directed mutagenesis, eukaryotic promoters, cell culture, the use of plasmid or adenoviral vectors for silencing proteins, protein-protein interactions (e.g., GST pull-downs, ChIP assays), nuclear receptors, coactivators, and epigenetics is desirable. To apply, submit a curriculum vitae including a bibliography and the names of 3 academic references (with email addresses and telephone numbers) to:

Dr. Joyce Goldstein
National Institute of Environmental Health Sciences
PO Box 12233, Maildrop A3-02,
Research Triangle Park, NC 27709
E-mail: goldste1@niehs.nih.gov



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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



WWW.NIH.GOV

NIAID Transition Program in Clinical Research

The NIAID Transition Program in Clinical Research (TPCR) provides an opportunity for physicians to gain clinical and translational research experience in the NIAID Division of Intramural Research (DIR). The program aims to increase the pool of well-trained clinical investigators who are competitive for clinical tenure-track positions. This program is targeted to physician investigators who have completed specialty training but not yet received independent funding. The TPCR award represents an opportunity to gain progressive training leading to scientific independence in a mentored environment.

Up to three candidates per year will be selected for three- to five-year appointments as assistant clinical investigators. Applicants must have an M.D. or M.D./Ph.D., be board eligible or board certified in a subspecialty (or equivalent), and qualify for credentialing by the NIH Clinical Center. Applicants should identify a DIR laboratory chief who will agree to host their research. Information about DIR laboratories and contact information for laboratory chiefs is

available at www.niaid.nih.gov/about/organization/dir/.

Applications will be evaluated by a search committee composed of NIAID intramural principal investigators with clinical and basic research interests. Competitive candidates will be asked to present their research accomplishments and plans to the search committee.

Participants will receive independent resources and staff and will be mentored by an NIAID senior clinical investigator.

Interested candidates may contact DIR Deputy Director Dr. Karyl Barron at 301-402-2208 or kbarron@nih.gov for additional information or assistance in identifying an appropriate host lab.

To apply for the program, e-mail curriculum vitae/bibliography, a research program proposal (no more than two pages), and a letter of support from the accepting NIAID lab chief by **October 15, 2010** to Ms. Bao-Hanh Ngo at NIAIDTPCR@mail.nih.gov. In addition, three letters of recommendation must be sent directly from the referees to Dr. Karyl Barron, Chair, Transition Program in Clinical Research Search Committee, c/o Ms. Bao-Hanh Ngo, by e-mail at NIAIDTPCR@mail.nih.gov or by post at 10 Center Drive, MSC 1356, Building 10, Room 4A-22, Bethesda, MD 20892-1356. E-mail is preferred. Please note search #029 when sending materials.

Further information about working at NIAID is available on our Web site at www.niaid.nih.gov/careers/tpnm.

NIAID

National Institute of Allergy and Infectious Diseases



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health



National Institute of Allergy and Infectious Diseases

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THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tenure-track Faculty Position(s) in Chemistry

The Department of Chemistry invites applications for two or possibly three tenure-track faculty positions (preferably at the rank of Assistant Professor). Preference will be given to applicants in the areas of organic and analytical chemistry.

The appointees are expected to teach at both undergraduate and postgraduate levels, and to develop a vigorous research program in their area of interest.

Applicants should have a PhD degree in chemistry or related fields and relevant postdoctoral experience. The starting salary will be commensurate with qualifications and experience. Fringe benefits including medical/dental benefits and annual leave will be provided. Housing will also be provided where applicable. Initial appointment will normally be on a three-year contract, renewable subject to mutual agreement. A gratuity will be payable upon successful completion of contract.

Applications including a curriculum vitae, a statement of teaching philosophy, a research proposal, and names and addresses of three referees (including email addresses) should be sent to:

Chemistry Faculty Search Committee
Department of Chemistry
The Hong Kong University of Science and Technology
Clear Water Bay, Kowloon, HONG KONG
Fax: (852) 3521-1486
Email: chemsearch@ust.hk

The review of the applications will start on 15 September 2010 until the positions are filled.

Additional information about the University and the Department is available at <http://www.ust.hk>.

(Information provided by applicants will be used for recruitment and other employment-related purposes.)



University of Connecticut Health Center

Chair of the Department of Cell Biology

The University of Connecticut Health Center's School of Medicine seeks a highly qualified individual with an outstanding record of accomplishment in research, service, and education to become Chair of the Cell Biology department. The successful candidate will be an energetic and visionary individual who is presently at the Associate or Full Professor level and shows evidence of the leadership skills required to establish and implement a strategic vision for the department. The Chair will build on existing strengths in cell analysis and modeling, vascular biology, and reproductive biology to meet current needs and capitalize on emerging opportunities. The administration is strongly committed to growing the department to enhance both the research portfolio and the contribution to the preclinical medical school curriculum. In addition to oversight responsibility for the research, education, mentoring, and administrative (including budgetary) activities of the department, the Chair will play a major role in governance at the institutional level.

The University of Connecticut Health Center is a vibrant organization composed of the School of Medicine, the School of Dental Medicine, the Graduate School of Biomedical Science, the John Dempsey Hospital and the UCONN Medical Group. The Health Center's campus is situated on 162 acres of wooded hilltop in the beautiful, historic community of Farmington, Connecticut.

Candidates should apply by submitting a curriculum vita and the names of three references via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>, search no. 2011-070, or directly via email to the search committee chair, Dr. Barbara Kream at cellbiochair@uchc.edu

UCHC is an Equal Opportunity Employer M/F/V/PwD



Department: Center in Molecular Toxicology and the Vanderbilt Institute for Clinical and Translational Sciences

Position: Integrative Health Sciences Facility Core Research Program Coordinator. The Center in Molecular Toxicology and the Vanderbilt Institute for Clinical & Translational Research at Vanderbilt University School of Medicine seek candidates for a Research Assistant Professor or Research Associate Professor position in the non-tenure research track to lead a newly created and novel Integrative Health Sciences Facility Core, the goals of which are to promote and develop translational research in the environmental health sciences. Applicants must have a Ph.D., M.D., or equivalent degree, and have demonstrated excellent qualifications in research, preferably in environmental sciences. In addition, specific expertise in research project coordination (i.e., research design and project organization, collaboration, basic analytic skills, and program development, etc.), and manuscript and grant writing experience are essential. Excellent verbal and written communication skills, as well as the ability to work in a team are essential.

This is an ideal position for someone who may desire to transition from being a PI to a Research Associate Professor or Research Professor, with the opportunity to lead and facilitate new and novel research directions and opportunities for funding in the environmental sciences. Significant infrastructure resources exist to develop unique integrative and translational research programs with world-renowned scientists.

Please submit curriculum vitae, and a letter of interest to:

Tina V. Hartert, MD, MPH
Vanderbilt University School of Medicine
6107 Medical Center East
Nashville, TN 37232-8300
(615) 936-1010
tina.hartert@vanderbilt.edu

Vanderbilt University School of Medicine is an equal opportunity, affirmative action employer. Women and minority candidates are strongly encouraged to apply.



Physicist Sector 3

The X-ray Science Division at Argonne National Laboratory invites applications for a staff position as beamline scientist at the insertion device beamline 3-ID at the Advanced Photon Source. We are seeking candidates in experimental x-ray science, with particular emphasis on nuclear resonant scattering and inelastic x-ray scattering. The position may be filled at an entry or a more senior level.

Candidates should have a strong background in synchrotron radiation research; considerable skills in developing x-ray instrumentation, in particular for nuclear resonant spectroscopy; combined with the ability to understand abstract concepts and synthesize scientific results in the experimental and theoretical framework of the field. Experience with hardware and software for computer control and data collection are beneficial and good oral and written communication skills are a must.

The Advanced Photon Source at Argonne provides a stimulating intellectual environment, and offers the successful candidate many opportunities to interact with world-class researchers and facilities.

The successful candidate will have a Ph.D., postdoctoral experience and a demonstrated ability to conduct high-quality independent research in the field.

Argonne is a U.S. Department of Energy laboratory managed by UChicago, LLC. Argonne's site is approximately 25 miles southwest of Chicago on a beautiful 1500-acre campus-like environment. Interested candidates should send a detailed CV, along with a list of publications and the names and addresses of three references through the Argonne website <http://www.anl.gov/jobs> for the following requisition:

316252 (Physicist)

For additional technical information, please contact Dr. Thomas Gog at gog@anl.gov or Dr. Ercan Alp at eea@aps.anl.gov.

Argonne is an equal opportunity employer, and we value diversity in our workforce.

UNIVERSITY PROFESSOR POSITIONS

The Pohang University of Science and Technology (POSTECH) is Korea's first research-oriented university and is known for its unique contribution to the advancement of science and technology in Korea. POSTECH is ranked first among Asia's specialized universities in the 2010 Chosun Ilbo - QS Asian University Rankings.

POSTECH invites distinguished scholars to join in POSTECH's efforts to become a global leader in science & technology research.

Position Announcement:

We are seeking distinguished scholars in all fields of science and engineering. Appointments will be made for multiple positions at the rank of University Professor. POSTECH will provide startup funds of up to 7 million US dollars for each distinguished scholar. Salary will be competitive and commensurate with candidate's qualifications and experience. The exact amount of financial support may vary depending on the field.

Desired Qualifications:

- Nobel laureates, Fields medalists or scholars of equivalent caliber
- Members of world-renowned national academies of sciences/engineering such as the National Academy of Sciences (USA), the National Academy of Engineering (USA), and the Royal Society (UK)
- Editors of the world's top scientific journals or those with equivalent experience
- Those who have demonstrated potential to grow to become a University Professor are also welcome to apply.

Interested individuals should send a CV, a statement of research plans, and a teaching statement to **e-mail: recruit@postech.ac.kr** or **mailing address: Faculty Search Committee, Office of Academic Affairs, POSTECH, San 31, Hyoja-dong, Nam-gu, Pohang 790-784, Republic of Korea.**

ASSOCIATE PROVOST AND DEAN FOR SCIENCES THE GRADUATE CENTER OF THE CITY UNIVERSITY OF NEW YORK

The Graduate Center of the City University of New York invites nominations and applications for the position of Associate Provost and Dean for Sciences. Reporting to the Provost and Senior Vice President for Academic Affairs and serving as a member of the President's cabinet, the Associate Provost and Dean for Sciences will provide vision and leadership in shaping and advancing the Graduate Center's strategy in the Sciences.

The Graduate Center is the doctorate-granting institution of the City University of New York (CUNY). An internationally recognized center for advanced studies and doctoral education, it enrolls more than 4,600 students in 34 doctoral and 8 master's programs in the humanities, social sciences and sciences. The Graduate Center offers doctoral programs in computer science, earth and environmental science, linguistics, mathematics, psychology, speech-language-hearing sciences, and the health sciences (audiology, nursing, physical therapy and public health). It also offers doctoral programs in biochemistry, biology, chemistry, and physics jointly with City College and Hunter College. Several initiatives in the sciences have begun to take shape. One, in the Theoretical Sciences, was launched in February of 2010 with a series of colloquia and public lectures that have attracted renowned theorists and scientists to the Graduate Center. Another, funded through a substantial multiple-year grant from the Mellon Foundation and resources from the University, is establishing three interdisciplinary committees – in science studies, religion, and globalization. These committees will enhance faculty collaboration and create synergy between the humanities, the social sciences, and the sciences.

The Associate Provost and Dean for Sciences will be responsible for overseeing the science doctoral programs and the joint doctoral degree programs in addition to associated five-year student fellowship packages and the health science programs. S/he will manage a broad range of administrative and fiscal matters and also be responsible, together with the Associate Provost and Dean for the Humanities and Social Sciences, for providing leadership in interdisciplinary initiatives and science studies.

Qualifications: A doctoral degree and record of distinguished scholarly achievement qualifying for a tenured appointment in one of the doctoral programs in the sciences (including computer science and mathematics); a record of significant senior administrative experience; a demonstrated ability to interact effectively and work collaboratively with faculty and program heads in promoting research and programs that advance academic excellence in the sciences; commitment to the principles of a large, urban public university; a broad understanding of and enthusiasm for the highest standards of academic achievement both within the disciplines and in innovative combinations of them; the ability to think creatively; good problem-solving skills; a high level of professional and personal integrity; a proven ability to manage human and financial resources; and a talent for dealing creatively and flexibly with people and issues.

For more information about The Graduate Center and this position, please visit <http://www.gc.cuny.edu> or <http://web.gc.cuny.edu/HumanResources/job-listings/gc-listings.htm>.

For full consideration, applications should be postmarked no later than September 10, 2010.

Applicants should provide: 1) letter of application; 2) curriculum vitae; 3) names/addresses/telephone numbers/email addresses of five professional references (who will not be contacted without applicant's prior approval). Nominators should send a letter of nomination and nominee's curriculum vitae. All materials should be addressed to:

CUNY Graduate Center
Search, Associate Provost and Dean for Sciences
Office of the Provost, Room 8113
365 Fifth Avenue
New York, NY 10016

CUNY is an EO/AA/ADA/IRCA Employer.

THE GRADUATE CENTER IS 



HD Biosciences Co. Ltd., is a leading biology focused preclinical drug discovery CRO based in Shanghai. The company offers comprehensive technology platforms and expertise from Target to Hit, Hit to Lead and Lead Op in drug discovery, both small molecules and biologics. The company's strategic clients include Pfizer, Lilly, Merck, and many other pharmaceutical and biotech companies. The outstanding track records of services and capability to provide inputs in the projects have helped company to earn great reputation in the industry.

As part of our growing R&D business, we are recruiting to fill several key positions. We are searching for qualified, self-motivated and experienced individuals with strong capability and track record in the following areas:

- ◆ Vice President/Head, Business Development
- ◆ Head/Senior Director/Director/Principal Scientist, Integrated Drug Discovery and *in vitro* pharmacology
- ◆ Senior Director/Director/Principal Scientist, *In vivo* Pharmacology
- ◆ Director/Principal Scientist, Toxicology
- ◆ Director/Principal Scientist, DMPK
- ◆ Director/Principal Scientist/Senior Scientist/Scientist, Discovery Biology in Enzyme, Receptor, and Ion Channel

We provide excellent career development opportunities, competitive compensation package and employee stock option plan to qualified employees. We are proud to be an Equal Opportunity Employer. To learn more about HDB or view a complete list of job openings and apply online, please visit us at: www.hdbiosciences.com or submit a copy of curriculum vitae to recruiting@hdbiosciences.com.

HD Biosciences Co., Ltd. Phone: +86-21-51163700
590 Ruiqing Road, Zhangjiang East Campus, Pudong, Shanghai 201201, P. R. China
Email: operation@hdbiosciences.com



CHAIR OF THE DEPARTMENT OF MECHANICAL ENGINEERING AND MATERIALS SCIENCE

Duke University and the Pratt School of Engineering invite applications and nominations for the position of Chair of the Department of Mechanical Engineering and Materials Science (MEMS). MEMS is one of four departments in the Pratt School of Engineering, within a world-class, top-ranked teaching and research institution.

The Department currently has 26 full-time faculty members and over 250 students pursuing B.S., M.S., or Ph.D. degrees in Mechanical Engineering and Materials Science. With average annual research expenditures of \$6M, the Department was recently ranked 8th nationally for faculty productivity (Chronicle of Higher Education, 2008). The Department derives strength from cross-disciplinary collaborations in the School of Engineering, in the School of Medicine, and in Duke's hallmark interdisciplinary centers.

Successful candidates will have a track record of dynamic academic leadership skills, possess excellent communication and interpersonal skills, and be able to lead the Department in developing and implementing a compelling vision for its research, education, and service programs. Key responsibilities will include strategic planning, development of emerging areas, coordination of interdisciplinary activities, and assistance with securing funding for scholarships, endowed chairs, and center/focus area initiatives.

Candidates must possess a Ph.D. or equivalent in Mechanical Engineering, Materials Science, or a closely related field, and must qualify as a full professor in the department, able to continue a productive research program of international stature. The preferred start date for this position is July 2011. Applicants should submit the application containing a cover letter, complete curriculum vitae, and names and addresses of at least four references to the following website: <http://www.mems.duke.edu/application-for-chair>.

Please contact the head of the search committee, **Prof. Stefan Zauscher** (zauscher@duke.edu) with questions or nominations of candidates. Applications received before **November 1, 2010** will receive full consideration, but applications will continue to be accepted until the position is filled.

Duke University and Health System is an Equal Opportunity Institution. Duke is committed to recruiting, hiring, and promoting qualified minorities, women, individuals with disabilities, and veterans.

Neutron Scattering Instrument Development Leader

Neutron Sciences Directorate at Oak Ridge National Laboratory invites applications for a Instrument Development Leader. ORNL is becoming the world's foremost center for neutron science. Research at ORNL encompasses the physical, chemical, materials, biological, and medical sciences and will provide opportunities for up to 2000 researchers each year from industry, research facilities, and universities all over the world.

We seek a candidate who can lead the effort to provide cutting-edge scientific capabilities at SNS and HFIR through the continued modernization of operating instruments, the development of new techniques on existing instruments, the development of new instruments, and development of instruments for new sources such as the SNS second target station and the HFIR second cold source. Must have an understanding of the scientific contributions that can be made with neutron scattering with the vision to identify and lead important new thrusts in instrumentation to enhancing neutron scattering scientific capabilities.

For more information about the position or to apply visit: http://jobs.ornl.gov/neutron_science.shtml

jobs.ornl.gov

To learn more about neutron science at ORNL visit: <http://neutrons.ornl.gov/>



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University of Glasgow

College of Medicine, Veterinary & Life Sciences
Beatson Institute

Lecturer/Senior Lecturer/Reader

Ref: 00462-1 (Senior Lecturer) Ref: 00463-1 (Lecturer)

The Glasgow Centre for Cancer Research, comprising the Beatson Institute for Cancer Research and the University of Glasgow, is engaged in a programme of world-class science directed towards understanding aspects of cancer cell growth, motility and survival. As part of an ongoing expansion, we are seeking applications from successful and highly motivated scientists to join us as a Junior Group Leader. We particularly welcome applicants using mouse models of cancer to develop an independent research programme in one of our key research areas. You should have a minimum of three years' postdoctoral training and an excellent publication record.

The GCCR is housed in a complex of new buildings that provide an outstanding environment for our scientists. We offer generous support that includes a competitive personal salary, a start up package that will allow recruitment of up to 5 additional positions, running costs, high quality lab space with room for expansion and access to state-of-the-art facilities and core services. In total this package of support is worth approximately £1million. Further information on the Beatson's research activities, infrastructure and facilities is available on our website www.beatson.gla.ac.uk

Informal enquiries can be directed to Professor Karen Vousden, Director, the Glasgow Centre for Cancer Research and the Beatson Institute for Cancer Research, (Email: k.vousden@beatson.gla.ac.uk).

Apply online at www.glasgow.ac.uk/jobs

If you are unable to apply online please contact us on 0141 330 3898 for an application pack.

Closing date 31 October 2010.

The University is committed to equality of opportunity in employment. The University of Glasgow, charity number SC004401.



www.glasgow.ac.uk



University of Connecticut – Storrs

FACULTY POSITION - STRUCTURAL BIOLOGY AND BIOPHYSICS

The Department of Molecular and Cell Biology at the University of Connecticut invites applications for a 9-month **TENURE-TRACK ASSISTANT PROFESSOR** (Search # 2011088) in the area of Structural Biology and Biophysics starting August 23, 2011. The Department of Molecular and Cell Biology has a vibrant research environment for Structural Biology and Biophysics (<http://biophysics.uconn.edu>). The successful candidate will be expected to develop a productive, independent research program, teach at the undergraduate and graduate levels and employ structural and biophysical approaches to address important biological problems.

Minimum qualifications include a Ph.D. in Biophysics, Biochemistry or a closely related field, postdoctoral experience and an outstanding record of research accomplishments. Equivalent foreign degrees are acceptable. Preferred qualifications include the ability to contribute through research, teaching, and/or public engagement to the diversity and excellence of the learning experience. This position is at the main campus in Storrs; however, incumbents may also work at the University of Connecticut's regional campuses in Avery Point, Hartford, Stamford, Torrington, Waterbury and West Hartford. Applicants should login at <http://www.jobs.uconn.edu> to submit a CV and a concise statement of research and teaching interests. Please also arrange to have three letters of recommendation sent to: **Chair, Structural Biology and Biophysics Search Committee, Department of Molecular and Cell Biology U-3125, University of Connecticut, Storrs, CT 06269-3125**. Review of applications will begin upon publication of this ad and continue until the position is filled.

FACULTY POSITION – CELL AND DEVELOPMENTAL BIOLOGY

The Department of Molecular and Cell Biology at the University of Connecticut seeks applicants for a 9-month tenure-track position at the level of **ASSISTANT PROFESSOR** (Search # 2011086) in the area of Cell and Developmental Biology, starting August 23, 2011. We are particularly interested in candidates working in the areas of: signal transduction, stem cells, cell motility, developmental processes, cancer biology and host-pathogen interactions. The Department has strengths in the fields of cellular stress responses, immune cell signaling, cytoskeleton and motility, muscle differentiation, regulation of gene expression and cancer biology. Additional information on the Cell Biology group can be found at the departmental web site (<http://mcb.uconn.edu/>). The successful candidate is expected to establish a productive research program, and to teach in the undergraduate and graduate program in cell biology.

Minimum qualifications include a PhD in Cell and Developmental Biology or closely related field; postdoctoral experience; an outstanding research record. Equivalent foreign degrees are acceptable. Preferred qualifications include the ability to contribute through research, teaching, and/or public engagement to the diversity and excellence of the learning experience. This position is at the Storrs Campus, although incumbents could also work at the University of Connecticut's campuses at Avery Point, Hartford, Stamford, Torrington, Waterbury, and West Hartford. The University of Connecticut, ranked first among public universities in New England, is located in northeastern Connecticut with easy access to Boston and New York City. Applicants should login at <http://www.jobs.uconn.edu> to submit a CV, concise statements of research and teaching interests, and the names and contact information of three people who have agreed to provide letters of recommendation. Review of applications will begin upon publication of this ad and continue until the position is filled.

The University of Connecticut is an EEO/AA Employer.



ASSISTANT/ASSOCIATE PROFESSOR UNIVERSITY OF NEBRASKA-LINCOLN (UNL) DEPARTMENT OF BIOCHEMISTRY

The **Department of Biochemistry** continues to expand its faculty in basic research and invites nominations and applications for a **Plant Biochemist** using algae as a prime model system at the Assistant or Associate Professor level. This academic-year position will be supported in part by a National Science Foundation Experimental Program to Stimulate Competitive Research (EPSCoR) Grant. We seek a broadly trained biochemist who will conduct vigorous basic research in modern metabolic biochemistry and molecular biology using algae as a model system to address significant metabolic processes such as complex lipid metabolism, integration of metabolomics with gene and metabolic regulation, or the metabolic challenges related to production of biofuels or other high-value products in algae. The successful candidate will be part of an interdisciplinary team that will contribute essential basic information on eukaryotic green algae and how it can be exploited as in biofuel production. This position is part of the strategic plans of UNL, the Institute of Agriculture and Natural Resources, and Department of Biochemistry directed to strengthen the molecular life sciences. This position is housed in the George W. Beadle Center, which includes state-of-the-art core facilities in proteomics and metabolomics, genomics, crystallography, bioimaging, flow cytometry, bioinformatics, biophysical spectroscopy, and trace element analysis. It is expected that the incumbent will have (Associate Professor) or establish (Assistant Professor) a nationally recognized and extramurally funded research program and contribute to the undergraduate and graduate teaching missions of the Department of Biochemistry.

A PhD (or equivalent) in biochemistry or related field and two years postdoctoral experience (or equivalent) is required. Candidates applying for this position at the Associate Professor level must have an established externally funded research program and time-in-rank (minimum of five years) at the Assistant Professor (or equivalent) level. This position comes with a highly competitive start-up package. Lincoln Nebraska boasts an outstanding quality of life that includes fine culinary and artistic treasures, a budding live music scene and numerous parks, golf courses and bike trails. In 2008, WebMD reported that Lincoln was the healthiest city in the United States.

To learn more about the University of Nebraska, the Biochemistry Department, visit <http://biochem.unl.edu>. Applicants should go to <http://employment.unl.edu>, search for requisition number **100474**, complete the Faculty Academic/Administrative Information form, attach a letter of application, Curriculum Vitae, a statement of research, and statement of teaching interests. Applicants must arrange for three confidential letters of reference to be sent directly to: **Search Committee, Department of Biochemistry, University of Nebraska, N200 Beadle Center, Lincoln, NE 68588-0664, USA** or by e-mail to biochemistrysearch@unl.edu. Review of applications will begin on **October 15, 2010** and continue until the position is filled or the search is closed.

The University of Nebraska has an active National Science Foundation ADVANCE gender equity program, and is committed to a pluralistic campus community through affirmative action, equal opportunity, work-life balance, and dual careers.

Upcoming Faculty Issue



Issue date:
September 10
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FRED HUTCHINSON CANCER RESEARCH CENTER A LIFE OF SCIENCE

The Division of Basic Sciences invites applications at the **Assistant Member** level. We are seeking outstanding scientists conducting cutting-edge research into fundamental biological questions. Current faculty members investigate diverse areas of genetic, molecular, cellular, metabolic, developmental, evolutionary and structural biology and utilize a broad range of approaches and experimental systems. We support our junior faculty by providing a collegial environment and a wealth of technical resources. The Fred Hutchinson Cancer Research Center is located in a modern campus by Lake Union in Seattle, Washington, and is close to other non-profit research institutes and the University of Washington. It has an outstanding graduate program and state of the art facilities and research resources. Further information about the Center is available at <http://www.fhcr.org/basic>.

Candidates should visit
<http://www.fhcr.org/basicsearch>
for application instructions.

Application deadline: **November 12, 2010**

The Fred Hutchinson Cancer Research Center is an Equal Opportunity Employer, committed to workforce diversity.



MOUNT SINAI
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Tenure-track Faculty Positions in Immunology

The Mount Sinai Immunology Institute is seeking outstanding scientists to develop rigorous independent research programs in basic or translational immunology. Applicants whose programs address inflammatory bowel disease, gut-microbiome interactions, mucosal immunity, and intravital imaging are encouraged to apply. The Immunology Institute is a diverse, interactive group interested in basic and translational immunology and mechanisms of immune-mediated diseases, <http://research.mssm.edu/iisinai>. Applicants may have an MD, PhD or MD/PhD degrees and may be appointed at the Assistant, Associate or Full Professor levels, at Mount Sinai School of Medicine with a generous start-up package.

Applicants should submit a CV, research plan and the names of three references to: **Immunology Institute Faculty Search Committee**, email: Zaida.Newman@mssm.edu. Review of applications will begin **September 2010**.

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ScienceCareers.org/booklets



Science Careers

From the journal *Science*





Vice President for Facilities and Operations The Research Institute at Nationwide Children's Hospital

The Research Institute at Nationwide Children's Hospital in Columbus, Ohio, is seeking an outstanding leader to oversee scientific operations, facility planning, core laboratory services, laboratory animal facilities, and research education.

Candidates should have significant executive level experience in a biomedical research institution, university, academic enterprise or biotechnology company. A master's or PhD degree in finance, business, life science or health services is preferred. Practical knowledge of research laboratory operations and management, shared research resources and instrumentation, bioinformatics, finance, budgeting, strategic planning, and research compliance and regulations are attractive attributes for this leadership position.

The Research Institute at Nationwide Children's Hospital administers and supports all research activity on our campus. Extensive scientific collaboration and intellectual exchange occurs with The Ohio State University main campus. The Research Institute is a top 10 National Institutes of Health-funded free-standing pediatric research center. A \$93 million, six-floor world-class research facility, which will add 225,000 square feet to the current 320,000 square feet dedicated to research on the Nationwide Children's campus, is slated to open in 2012. The Research Institute is expected to attract in excess of \$300 million in external support over the next five years, making it one of the fastest growing pediatric research institutes in the nation.

For more information, please visit
www.nationwidechildrens.org/research.

Interested potential candidates should contact
Toni Davis, DHR International, (678) 385-5008
or tdavis@dhrinternational.com

Nationwide Children's Hospital and The Ohio State University are equal opportunity employers that value diversity. Candidates of diverse backgrounds are encouraged to apply.



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WELCOMING AND WORLD-CLASS

In light of the importance of research to improving healthcare, Winthrop-University Hospital's vision for the future includes our new Research Institute. Rivaling those in top national academic medical centers, our new environment will nurture creative scientific thought and includes state-of-the-art laboratories, as well as comprehensive support and core services.

RESEARCH SCIENTISTS - LONG ISLAND, NY -

DIABETES & OBESITY RESEARCH CENTER

The Winthrop-University Hospital Research Institute is actively recruiting MD or PhD scientists to join the current group of clinical and basic researchers in the development of a new translational *Diabetes and Obesity Research Center*.

The successful candidate will be capable of developing an innovative program of research focused on the causes and complications of diabetes and obesity, as well their prevention, treatment and cure.

BONE AND MINERAL METABOLISM

The Winthrop-University Hospital Research Institute is actively recruiting a MD or PhD scientist to join the current group of clinical and basic researchers studying bone and mineral metabolism.

The successful candidate will be capable of developing an innovative program of research focused on the causes and complications of osteoporosis, and/or the systemic effects of bone metabolism as they pertain to the development and/or progression of other diseases, such as diabetes.

For the above positions, it is expected that senior candidates will have an established track record of and existing funding and junior candidates will have a high degree of potential for becoming successful independent investigators. Appointments from the Assistant to Professor level will be made commensurate with the level of academic achievements.

As a major teaching affiliate of Stony Brook University, Winthrop provides a vibrant, interactive environment offering numerous opportunities for multidisciplinary collaborative investigations. Construction of a *new research facility* and a *generous recruitment package* including support for a team of researchers in clinical, basic, translational and outcomes research, create exceptionally exciting and unique opportunities.

Winthrop is located in suburban Long Island, NY, 30 minutes from midtown Manhattan on the LIRR and just minutes from LI's beautiful beaches.

Please send letter of interest & curriculum vitae, indicating position of interest, to: **Alan M. Jacobson, MD, Chief Research Officer, Winthrop-University Hospital, 222 Station Plaza North, Ste 300, Mineola, NY 11501. Email: amjacobson@winthrop.org**

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POSITIONS OPEN

BIOMOLECULAR NMR SPECTROSCOPIST Indiana University

Indiana University seeks to appoint a biomolecular nuclear magnetic resonance (NMR) Spectroscopist at the **ASSISTANT SCIENTIST** (research-track) level, effective January 1, 2011 to oversee operation of 600 and 800 MHz Varian spectrometers in the METACyt Biomolecular NMR Laboratory. Candidates must have a Ph.D. in chemistry, biochemistry, structural biology, or a related field; two years postdoctoral experience or equivalent is desired. The successful candidate will have experience with the application and optimization of modern biological NMR spectroscopy to the study of proteins and protein complexes. Experience with the operation and maintenance of high field NMR spectrometers and cryogenic probe systems is required, and knowledge of hardware and Unix-based software maintenance is essential. Familiarity with Varian DirectDrive systems, VnmrJ, and methods of NMR data analysis are desirable but not required.

Applicants should submit a brief statement of interest, curriculum vitae, and arrange for three letters of recommendation to be sent to: **Professor David P. Giedroc, Chair of Search Committee, Department of Chemistry, Indiana University, 800 East Kirkwood Avenue, Bloomington, IN 47405. Fax: (812)-856-5050, e-mail: chemchair@indiana.edu.** Applications received by October 15, 2010 will be assured consideration; however, review of applications will begin upon receipt and will continue until the position is filled. *Indiana University is an Equal Opportunity/Affirmative Action employer.*

FACULTY POSITIONS

Microbiology and Immunology

University of North Carolina at Chapel Hill

The Department of Microbiology and Immunology in the School of Medicine at the University of North Carolina (UNC) at Chapel Hill invites applications for tenure-track faculty from individuals studying innate immunity of host immune response to pathogens, inflammation, and/or cancer. Applicants at the **ASSISTANT PROFESSOR, ASSOCIATE PROFESSOR, OR PROFESSOR** level will be considered.

UNC-Chapel Hill has a strong collaborative environment that includes basic, clinical, and translational research. Successful applicants will have opportunities to participate in larger interdisciplinary studies while developing an independent research program and participating in teaching. Information about the Department of Microbiology and Immunology is available at **website: <http://www.med.unc.edu/microimm/>.**

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. Please apply online at **website: <http://jobs.unc.edu/1002239>** by submitting individual PDF files containing: (i) curriculum vitae including contact information for three references, (ii) a cover letter, and (iii) a one- to two-page description of future research plans. Review of applications will begin October 1, 2010.

The University of North Carolina at Chapel Hill is an Equal Opportunity Employer.

POSTDOCTORAL RESEARCH FELLOWS The Methodist Hospital Research Institute

Applications are invited for two Postdoctoral Research Fellows at The Methodist Hospital Research Institute, Molecular Imaging Program, to investigate novel targets of human disease, predominantly in cancer research. Our laboratory is focused on the characterization of novel proteins and pathways involved in oxidative stress response, cell signaling, and tumor progression. The successful candidate will have a Ph.D. in Biomedical Sciences with relevant publications in the field. Expertise in general molecular and cellular biology, knockout and transgenic animals, and in vivo tumor models is mandatory. Proficiency in biochemistry, protein-protein interaction, RNAi, protein phosphorylation analysis, proteomics, and molecular imaging are all highly desired. The position is immediately available for one year and can be reappointed to a NIH-funded position for subsequent years upon satisfactory progress of the project. Please send cover letter, curriculum vitae, and three references to: **Dr. Zheng-Zheng Shi via e-mail: zshi@tmhs.org.** *The Methodist Hospital System is an Equal Opportunity Employer.*

POSITIONS OPEN

VERTEBRATE BIOLOGIST

The Department of Biology of the College of the Holy Cross seeks an Organismal Biologist with expertise in vertebrates for appointment as a tenure-track **ASSISTANT PROFESSOR** beginning August 2011. Candidates must demonstrate commitment to, and excellence in, undergraduate teaching as well as scholarly achievement, and propose a research program involving undergraduates. A Ph.D. is required at the time of appointment and postdoctoral experience is desirable.

This position carries a 3-2 teaching load with a full-salary one-semester leave prior to tenure review and generous sabbatical and fellowship leaves for senior faculty. The appointee will be expected to develop an intermediate lab/field course on the biology of vertebrates or a vertebrate group, to develop a one-year human anatomy and physiology course targeted for non-premed pre-health students, and to participate in other teaching in the department.

Applicants should submit a cover letter, statements describing research interests and teaching philosophy, curriculum vitae, official academic transcripts, publications, and three letters of recommendation in hard copy by 15 October 2010 to: **Dr. Rob Bellin, Search Committee (Ref. S), Department of Biology, College of the Holy Cross, Worcester MA 01610.**

For detailed information on the position and application procedure, please see the Biology Department's **website: <http://academics.holycross.edu/biology/>.**

The College of the Holy Cross (enrollment 2,700) is a highly selective Catholic liberal arts college in the Jesuit tradition located in a medium-sized city 45 miles west of Boston. Holy Cross belongs to the Colleges of Worcester Consortium (**website: <http://www.cowc.org>**) and the New England Higher Education Recruitment Consortium (**website: <http://www.newenglandherc.org/home/index.cfm>**). *The College of the Holy Cross is an Equal Employment Opportunity Employer and complies with all Federal and Massachusetts laws concerning equal opportunity and affirmative action in the workplace.*

The Department of Internal Medicine seeks a faculty member at the **ASSISTANT PROFESSOR, ASSOCIATE PROFESSOR, or PROFESSOR** level. Potential for tenure and association is available dependent upon the applicant's qualifications. Rank and salary will be commensurate with qualifications/experience.

The qualified candidate will conduct research with a focus in cardiovascular medicine. Teaching and mentoring of postdoctoral fellows, junior research colleagues, or students at any level is expected. Grant writing and manuscript preparation is expected.

The candidate should possess a M.D. and/or Ph.D. and have at least three years of postdoctoral experience. Candidates should show ability to obtain external funding and evidence that they can conduct independent research.

Address inquiries to:

**The University of Michigan Medical Center
Department of Internal Medicine
Domino's Farms
24 Frank Lloyd Wright Drive
Lobby J, Suite 1200
Ann Arbor, Michigan 48106
Attn: Debbie Ventura**

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